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A MANUAL

OF

CLINICAL DIAGNOSIS

BY MEANS OF MICROSCOPIC AND CHEMICAL METHODS

FOR

STUDENTS, HOSPITAL PHYSICIANS, AND PRACTITIONERS

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SIXTH EDITION, THOROUGHLY REVISED

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J37
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TO

MY WIFE

WHO HAS SO FAITHFULLY AIDED IN ITS PREPARATION

THIS EDITION ALSO

IS

AFFECTIONATELY DEDICATED

PREFACE TO SIXTH EDITION.

In preparing the sixth edition of the *Clinical Diagnosis* the author was confronted with an important problem. A great deal of new material had to be introduced, but the size of the volume, which had steadily grown within the ten years of its existence, could not be exceeded. It was accordingly necessary to go over the entire work carefully and to cut out everything that was not of clearly practical value, to condense, and to rewrite. The amount of labor involved was considerable, but the object has been, it is hoped, satisfactorily achieved.

The chapter on the Blood has been further enlarged and brought thoroughly to date. Every page in the work has undergone a radical review. A new chapter on the Opsonins has been introduced, in which the subject-matter has been conservatively and, it is hoped, fairly presented; full details are given regarding the technical portion of the subject, in which the writer's experience as a pioneer worker may prove of value.

Two appendices have been added. The first deals with the preparation of culture media, and may prove of service to teachers who use the book not only as a text-book of clinical diagnosis, but also as a guide to the student's work in bacteriology. The second represents an outline of a course in clinical laboratory methods, and is presented at the request of teachers in clinical microscopy in many of our medical schools, in which the subject is steadily growing in importance. The "course" is based upon the work which the writer has conducted for post-graduates during the past ten years in his own laboratory, and is designed to be thoroughly practical and comprehensive.

Numerous illustrations in black and white, as well as a number of colored plates, mostly from the brush of Mrs. Simon, have been added.

The author wishes to thank the medical profession for the continued favorable reception of the *Diagnosis*, the pioneer work in America, and trusts that the present edition also will serve its purpose as a trustworthy guide to the medical student, general practitioner, and the laboratory research worker.

CHARLES E. SIMON.

1302 MADISON AVE., BALTIMORE,
1907.

CONTENTS.

CHAPTER I.

THE BLOOD.

PAGE
General considerations 17
General characteristics of the blood 17
  color 17
  odor 18
  specific gravity 18
    determination according to Hammerschlag 18
    determination according to Schmaltz and Peiper 19
    indirect estimation of the hemoglobin 19
  reaction 20
    estimation of the alkalinity according to Löwy 21
    estimation of the alkalinity according to Engel 21
    estimation of the alkalinity according to Dare 22
Chemical examination of the blood 25
  general chemistry of the blood 25
  coagulation of the blood 27
  blood pigments 29
    hemoglobin and oxyhemoglobin 29
    hemoglobinemia 33
    carbon monoxide hemoglobin 34
    nitric oxide hemoglobin 35
    hydrogen sulphide hemoglobin 35
    carbon dioxide hemoglobin 35
    hematin 35
    hemin 36
    methemoglobin 37
    hematoidin 38
    hematoporphyrin 38
  the proteids of the blood 39
  the carbohydrates 40
    sugar 40
      estimation of the sugar in the blood 41
      Williamson's diabetic blood test 42
    glycogen 42
    cellulose 42
  urea 42
    uremia 43
  ammonia 43
  uric acid and xanthin bases 43
  fat and fatty acids 45
  lactic acid 48
  biliary constituents 49
  acetone 50
  cholin 50
Microscopic examination of the blood 51
  the red corpuscles 51
    variations in the size of the red corpuscles 51
    variations in the form of the red corpuscles 52

Microscopic examination of the blood—*Continued.* PAGE
    variations in the color of the red corpuscles and color index . . 53
    variations in the number of the red corpuscles 54
    behavior toward aniline dyes (polychromatophilia) 60
    granular degeneration 61
    Cabot's ring bodies 64
    Ehrlich's hemoglobinemic Innenkörper 65
    nucleated red corpuscles 65
    normoblasts 65
    megaloblasts 67
  the leukocytes. 69
    general differentiation of the various forms of leukocytes . . . 69
    differentiation of the leukocytes according to their behavior toward aniline dyes 70
      the small mononuclear leukocytes 70
      the large mononuclear leukocytes 73
      the polynuclear neutrophilic leukocytes 74
      the polynuclear eosinophilic leukocytes 77
      the mast-cells 78
      the myelocytes 80
      Türck's irritation forms (phlogocytes) 82
      iodophilia 83
  leukocytosis 84
    polynuclear neutrophilic hyperleukocytosis 85
    polynuclear neutrophilic hypoleukocytosis 97
    neutrophilic karyomorphism 101
    polynuclear eosinophilic hyperleukocytosis 102
    polynuclear eosinophilic hypoleukocytosis 108
    lymphocytosis 109
    lymphopenia 112
    variations in number of the large mononuclear leukocytes. . . 112
    variations in number of the mast-cells 113
    myelocytosis 114
  the plaques 118
  the dust particles of Müller 120
General technique 120
  slides and cover-glasses 120
  the blood mount 121
  fixation 123
The aniline dyes and principles of staining 125
Methods of staining 129
  the eosinate of methylene blue 129
  Ehrlich's triacid stain 130
  the Romanowsky methods 132
    method of Hastings 132
    method of Leishman 134
    method of Wright 135
    method of Giemsa 136
    method of Goldhorn 137
Demonstration of iodophilia 137
Enumeration of the corpuscles of the blood 137
  method of Simon 137
    enumeration of the leukocytes 138
    enumeration of the red cells 140
    differential leukocyte counting 143
    enumeration of the plaques 144
The hematokrit 144
  volume index 147
Estimation of hemoglobin 147
  Dare's hemoglobinometer 148
  Fleischl's hemoglobinometer 150
  Gowers' hemoglobinometer (Sahli's modification) 152
  Talquist's method 152

PAGE
Estimation of blood iron 153
Kryoscopic examination of the blood 154
Osmotic resistance of the red cells 157
Bacteriology and parasitology of the blood 158
  typhoid fever 159
    agglutination test 160
  paratyphoid fever 165
  pneumonia 166
  pyogenic bacteriemia 168
  gonococcus septicemia 171
  anthrax 172
  acute miliary tuberculosis 173
  glanders 174
  influenza 174
  Malta fever 174
  bubonic plague 175
  malaria 177
  trypanosomiasis 187
  relapsing fever 189
  typhus fever 190
  Kala-azar 190
  syphilis 191
  spotted fever 191
  filariasis 191
  distomiasis (bilharziasis) 195
  anguilluliasis 196

CHAPTER II.

THE SECRETIONS OF THE MOUTH.

Saliva 198
  general characteristics 198
  chemistry of the saliva 198
  microscopic examination of the saliva 199
  pathological alterations 201
Special diseases of the mouth 202
    tuberculosis of the mouth 202
    actinomycosis 202
    catarrhal stomatitis 202
    ulcerative stomatitis 202
    gonorrheal stomatitis 202
    thrush 202
Tartar 203
Coating of the tongue 203
Coating of the tonsils 203
  pharyngomycosis leptothrica 203
  tonsillitis 204
  Vincent's angina 204
  diphtheria 205
  scarlatina 208
  glandular fever 208

CHAPTER III.

THE GASTRIC JUICE AND THE GASTRIC CONTENTS.

The secretion of gastric juice 209
Test meals 210
  the test breakfast of Ewald and Boas 210

Tests meals—*Continued.*

PAGE
- the test breakfast of Boas 210
- the test dinner of Riegel 210
- the double test meal of Salzer 210

The stomach tube 211
- contra-indications to the use of the tube 211
- introduction of the tube 211

General characteristics of the gastric juice 213
- amount 213

Chemical examination of the gastric juice 214
- the acidity of the gastric juice 214
- determination of the acidity of the gastric juice 215
- the amount of free hydrochloric acid 217
  - euchlorhydria 218
  - hypochlorhydria 218
  - anachlorhydria 218
  - hyperchlorhydria 218
- test for free acids 219
- test for free hydrochloric acid 219
  - the dimethyl-amino-azo-benzol test 219
  - the phloroglucin-vanillin test 220
  - the resorcin test 221
  - the tropeolin test 221
- the combined hydrochloric acid 222
- quantitative estimation of the hydrochloric acid 222
  - Töpfer's method 222
  - deficit of hydrochloric acid 223
  - Sahli's method 223
  - Martius and Lüttke's method 224
  - Leo's method 226
- the ferments of the gastric juice and their zymogens 227
  - pepsin and pepsinogen 227
    - tests for pepsin and pepsinogen 229
    - quantitative estimation 229
  - chymosin and chymosinogen 230
    - tests for chymosin and chymosinogen 231
    - quantitative estimation 232
- analysis of the products of albuminous digestion 232
- tests for the products of carbohydrate digestion 233
- lactic acid 233
  - mode of formation and clinical significance 233
  - tests for lactic acid 235
    - Kelling's test 235
    - Uffelmann's test 235
    - Strauss' test 236
    - Vournaso's test 236
    - Boas' test 237
  - quantitative estimation of lactic acid according to Boas' method 230
- the fatty acids 240
  - mode of formation and clinical significance 241
  - tests for butyric acid 241
  - tests for acetic acid 242
  - quantitative estimation of the fatty acids 242
- gases 244
- acetone 244
- vomited material 244
  - food material 246
  - mucus 246
    - gastrosuccorrhea mucosa 246
  - saliva 246
  - bile 246
  - pancreatic juice 247
  - blood 247

Chemical examination of the gastric juice—*Continued.* PAGE
test of Müller and Weber 247
Donogany's method 247
pus 248
stercoraceous material 248
parasites 249
odor 249
Microscopic examination of the gastric contents 249
the Boas-Oppler bacillus 250
sarcinæ 250
protozoa 251
shreds of mucous membrane 251
tumor particles 252
Examination of the motor power of the stomach 252
Leube's method 252
the salol test of Ewald and Sievers 253
Examination of the resorptive power of the stomach 253
Indirect examination of the gastric juice 254
Günzburg's method 254

CHAPTER IV.

THE FECES.

Examination of normal feces 256
general characteristics 256
number of stools 256
amount 256
consistence and form 257
odor 258
color 258
aloin test 260
guajac test 261
macroscopic constituents 261
alimentary detritus 261
mucous cylinders 262
concretions 263
foreign bodies 265
microscopic constituents 265
constituents derived from food 266
morphological elements derived from the alimentary canal . . 270
leukocytes 271
blood corpuscles 271
crystals 271
mucus 273
Bacteriology of the feces 273
Bacillus dysenteriæ 276
Bacillus typhi 278
Bacillus acidophilus 280
Bacillus vulgaris 281
Bacillus pyocyaneus 282
Bacillus coli communis 282
Bacillus lactis aërogenes 283
Bacillus choleræ 283
Bacillus tuberculosis 285
Animal parasitology 286
protozoa 286
Entamœba dysenteriæ 286
Entamœba coli 289
Paramœba hominis 289
Cercomonas hominis 290
Trichomonas intestinalis 291
Megastoma entericum 292

Animal parasitology—*Continued.* PAGE
  Balantidium coli . . . 293
  vermes . . . 295
    Tænia saginata . . . 295
    Tænia solium . . . 296
    Tænia nana . . . 297
    Tænia diminuta . . . 299
    Tænia cucumerina . . . 299
    Tænia Africana . . . 299
    Tænia Madagascariensis . . . 299
    Bothriocephalus latus . . . 300
    Krabbea grandis . . . 302
    Distoma hepaticum . . . 302
    Distoma lanceolatum . . . 302
    Distoma Buskii . . . 304
    Distoma sibiricum . . . 304
    Distoma spatulatum . . . 304
    Distoma conjunctum . . . 304
    Distoma heterophyes . . . 304
    Amphistomum hominis . . . 304
    Ascaris lumbricoides . . . 305
    Ascaris mystax . . . 307
    Ascaris maritima . . . 307
    Oxyuris vermicularis . . . 307
    Uncinaria duodenalis . . . 307
    Trichocephalus hominis . . . 311
    Trichina spiralis . . . 311
    Strongyloides intestinalis . . . 312
Chemistry of the feces . . . 316
  reaction . . . 316
  chemical composition . . . 317
  phenol, indol, skatol . . . 318
  fatty acids . . . 319
  cholesterin . . . 319
  biliary acids . . . 320
  pigments . . . 320
  purin bodies . . . 322
  mucin . . . 322
  albumin . . . 323
  albumoses . . . 323
  carbohydrates . . . 323
  ptomains . . . 323
Meconium . . . 323

CHAPTER V.

THE NASAL SECRETION.

Physiology and pathology of the nasal secretion . . . 325

CHAPTER VI.

THE SPUTUM.

General technique . . . 326
General characteristics of the sputa . . . 327
  amount . . . 327
  consistence . . . 327
  color . . . 328
  odor . . . 328
  specific gravity . . . 329
  configuration of sputa . . . 329

PAGE
Macroscopic constituents of sputum . . . 330
    cheesy particles . . . 330
    elastic tissue . . . 330
    fibrinous casts . . . 330
    Curschmann's spirals . . . 332
    echinococcus membranes . . . 333
    concretions . . . 333
    foreign bodies . . . 333
Microscopic examination . . . 334
    leukocytes . . . 334
    red blood corpuscles . . . 335
    epithelial cells . . . 336
    elastic tissue . . . 337
    animal parasites . . . 339
        Entamœba dysenteriæ . . . 339
        Trichomonades . . . 339
        Cercomonades . . . 339
        Tænia echinococcus . . . 339
        Distoma pulmonale . . . 343
        Distoma hematobium . . . 343
    vegetable parasites . . . 344
            the tubercle bacillus . . . 344
                methods of staining . . . 345
                    Gabett's method . . . 345
                    Weigert-Ehrlich's method . . . 346
                    Ziehl-Neelsen's method . . . 346
                cultivation of tubercle bacillus . . . 347
            the Diplococcus pneumoniæ . . . 348
            the bacillus of influenza . . . 349
            the bacillus of whooping-cough . . . 350
            the smegma bacillus . . . 351
            the typhoid bacillus . . . 351
            the plague bacillus . . . 351
            Micrococcus catarrhalis . . . 351
            Micrococcus tetragenus . . . 351
            staphylococci and streptococci . . . 351
            streptothrices . . . 351
                actinomycosis . . . 351
            blastomycetes . . . 353
            moulds . . . 353
        Oidium albicans . . . 354
        Sarcina pulmonalis . . . 354
        crystals . . . 354
            Charcot-Leyden crystals . . . 354
            hematoidin . . . 356
            cholesterin . . . 356
            fatty acid crystals . . . 356
            leucin and tyrosin . . . 357
            calcium oxalate . . . 357
            triple phosphates . . . 357
    the pneumoconioses . . . 357
        anthracosis . . . 357
        siderosis . . . 358
        chalicosis . . . 358
    stycosis . . . 358
Chemistry of the sputum . . . 358

CHAPTER VII.

THE URINE.

General characteristics of the urine . . . 360
    general appearance . . . 360
    color . . . 361

General characteristics of the urine—*Continued.*

PAGE

  odor 362
  consistence 362
  quantity 362
    polyuria 363
    oliguria 365
  specific gravity 366
    determination of the specific gravity 367
    determination of the solid constituents 368
Reaction 369
    determination of the acidity of the urine 371
Chemistry of the urine 373
  general chemical composition of the urine 373
  quantitative estimation of the mineral ash of the urine 373
  the chlorides 374
    test for chlorides in the urine 377
    quantitative estimation of the chlorides by the method of Salkowski-Volhard 377
    direct method 381
  the phosphates 382
    test for the phosphates in the urine 386
    quantitative estimation of the total amount of phosphates 387
    separate estimation of the earthy and alkaline phosphates 390
    removal of the phosphates from the urine 390
  the sulphates 390
    test for the sulphates in the urine 394
    quantitative estimation of the sulphates 394
      quantitative estimation of the total sulphates 394
      quantitative estimation of the conjugate sulphates 395
    neutral sulphur 396
  quantitative estimation 398
  urea 399
    properties of urea 407
    urea nitrate 408
    urea oxalate 408
    quantitative estimation of urea 409
      hypobromite method 409
      Folin's method 412
    estimation of nitrogen according to Kjeldahl 413
  ammonia 415
    quantitative estimation 416
      Folin's method 416
  uric acid 417
    properties of uric acid 423
    tests for uric acid 424
    quantitative estimation of uric acid (Folin's method) 424
  xanthin bases 427
    quantitative estimation (Salkowski's method) 427
  hippuric acid 429
    properties of hippuric acid 430
    quantitative estimation of hippuric acid 431
  kreatin and kreatinin 432
    properties of kreatin and kreatinin 433
    test for kreatinin in the urine 434
    quantitative estimation of kreatinin in the urine (Folin's method) 434
  oxalic acid 436
    properties of oxalic acid 438
    tests for oxalic acid 439
    quantitative estimation of oxalic acid (Dunlop's method) 439
Albumins 440
  serum albumin 441
  serum globulin 454
  albumoses 454

Albumins—*Continued*

PAGE

- Bence Jones' albumin 456
- peptone 457
- hemoglobin 458
- fibrin 459
- nucleo-albumin 460
- histon and nucleohiston 461
- tests for albumin 461
  - tests for serum albumin 462
  - nitric acid test 462
  - boiling test 464
  - potassium ferrocyanide test 465
  - trichloracetic acid test 465
  - picric acid test 466
  - Spiegler's test 466
  - special test for serum albumin 466
- quantitative estimation of albumin 467
  - old method of boiling 467
  - Esbach's method 467
  - gravimetric method 468
- test for serum globulin and its quantitative estimation 469
- tests for albumoses 469
  - Bang's method 470
- examination for peptone (polypeptids) 470
- tests for Bence Jones' albumin 471
- tests for (mucin) nucleo-albumin 472
- tests for hemoglobin 473
  - Heller's test 473
  - Donogany's test 474
- test for fibrin 474
- test for histon 474

Carbohydrates 474
- glucose 474
  - tests for sugar 483
    - Trommer's test 483
    - Fehling's test 484
    - Böttger's test with Nylander's modification 484
    - fermentation test 485
    - phenylhydrazin test 486
    - polarimetric test 487
  - quantitative estimation of sugar 489
    - Fehling's method 489
    - Gerrard and Allan's method 490
    - differential density method 491
    - Einhorn's method 492
    - Lohnstein's method 492
    - polarimetric method 494
- lactose 495
- levulose 495
- maltose 496
- dextrin 496
- laiose 496
- pentoses 496
  - Tollens' orcin test 497
  - Tollens' phloroglucin test 497

Glucuronic acid 497

Inosit 498

Urinary pigments and chromogens 498
- normal pigments 498
  - urochrome 499
  - uroerythrin 500
- normal chromogens 501
  - indican 501

Urinary pigments and chromogens—*Continued.*

PAGE

tests for indican 503
quantitative estimation 504
urohematin 506
uroroseinogen 507
pathological pigments and chromogens 508
blood pigments 508
hematin 508
urorubrohematin and urofuscohematin 508
urohematoporphyrin 508
biliary pigments 510
Smith's test 512
Huppert's test 512
Gmelin's test (as modified by Rosenbach). 512
Gmelin's test 512
biliary acids 512
cholesterin 513
pathological urobilin 513
melanin and melanogen 516
phenol 517
alkapton (homogentisinic acid) 518
blue urines. 521
green urines 521
pigments referable to drugs 521
Ehrlich's diazo reaction 522
benzaldehyde reaction 526
Acetone 527
tests for acetone 529
Legal's test 529
Lieben's test 529
Frommer's test 530
Dennigè's test 530
quantitative estimation. 531
Diacetic acid 532
Gerhardt's test 532
Arnold's test 533
Oxybutyric acid 533
estimation 534
Crotonic acid 535
Lactic acid 535
Oxyamygdalic acid 536
Volatile fatty acids 537
Amino-acids 538
Fat 539
Ferments 540
Gases 541
Ptomains 542
isolation of diamins 543
Kryoscopic examination of the urine 546
Sediments 546
Microscopic examination of the urine 546
non-organized sediments 549
sediments occurring in acid urines 549
uric acid. 549
amorphous urates 550
calcium oxalate 551
monocalcium phosphate 552
hippuric acid 552
calcium sulphate 553
cystin 553
leucin and tyrosin 554
xanthin 556
soaps of lime and magnesia 557

Microscopic examination of the urine—*Continued.*

PAGE

      - bilirubin and hematoidin . . . 557
      - fat . . . 557
    - sediments occurring in alkaline urines . . . 558
      - basic phosphate of calcium and magnesium . . . 558
      - neutral calcium phosphate . . . 559
      - magnesium phosphate . . . 559
      - ammoniomagnesium phosphate . . . 560
      - calcium carbonate . . . 560
      - ammonium urate . . . 560
      - indigo . . . 561
  - organized constituents of urinary sediments . . . 561
    - epithelial cells . . . 561
    - leukocytes . . . 563
    - red blood corpuscles . . . 567
    - tube casts . . . 570
      - examination . . . 571
      - true casts . . . 572
      - hyaline casts . . . 572
      - brown granular casts . . . 574
      - waxy casts . . . 574
    - pseudocasts . . . 576
    - cylindroids . . . 576
    - clinical significance of tube casts . . . 577
  - spermatozoa . . . 579
  - parasites . . . 580
    - vegetable parasites . . . 580
    - animal parasites . . . 586
  - tumor particles . . . 587
  - foreign bodies . . . 587

CHAPTER VIII.

TRANSUDATES AND EXUDATES.

- Transudates . . . 588
  - general characteristics . . . 588
  - specific gravity . . . 588
  - chemistry of transudates . . . 590
  - microscopic examination . . . 591
- Exudates . . . 591
  - serous exudates . . . 591
  - technique . . . 594
  - bacteriological examination of . . . 595
  - inoscopy . . . 596
  - chemistry of . . . 597
  - pus . . . 598
    - general characteristics of pus . . . 598
    - chemistry of pus . . . 598
    - microscopic examination of pus . . . 599
      - leukocytes . . . 599
      - giant corpuscles . . . 600
      - detritus . . . 600
      - red blood corpuscles . . . 600
      - pathogenic vegetable parasites . . . 600
      - protozoa . . . 601
      - vermes . . . 601
      - crystals . . . 602
      - technique . . . 602
      - gonorrheal pus . . . 602
        - the gonococcus . . . 603
  - putrid exudates . . . 604
  - chylous and chyloid exudates . . . 604

Exudates—*Continued.*

PAGE
syphilitic material 606
Spirochæte pallida 606

CHAPTER IX.

THE CEREBROSPINAL FLUID.

Amount 610
Appearance 610
Specific gravity 611
Reaction 612
Chemical composition 612
Microscopic examination 615
Cytology 615
Bacteriology 616
Toxicity 618

CHAPTER X.

THE EXAMINATION OF CYSTIC CONTENTS.

Cysts of the ovaries and their appendages 619
Hydatid cysts 621
Hydronephrosis 622
Pancreatic cysts 622

CHAPTER XI.

THE SEMEN.

General characteristics 623
Chemistry of the semen 623
Microscopic examination of the semen 624
Pathology of the semen 624
The recognition of semen in stains 625

CHAPTER XII.

VAGINAL DISCHARGES.

Bacteriology 627
Vaginal blennorrhea 628
Menstruation 649
The lochia 649
Vulvitis and vaginitis 629
Membranous dysmenorrhea 630
Cancer 630
Gonorrhea 630
Abortion 631

CHAPTER XIII.

THE SECRETION OF THE MAMMARY GLANDS.

The secretion of milk in the newly born 632
Colostrum 632
The secretion of milk in the adult female 633
Human milk 633
The milk in disease 634

The milk in disease—*Continued.* PAGE
determination of the specific gravity 635
estimation of the fat 637
estimation of lactose 637
estimation of the proteids 637

CHAPTER XIV.

THE OPSONINS.

The opsonins 639
Technique 642
The opsonic index 643

APPENDIX.

A.

Preparation of culture media 649

B.

Outline of a course in clinical microscopy 654
Lectures 654
Laboratory exercises 656

CLINICAL DIAGNOSIS.

CHAPTER I.

THE BLOOD.

GENERAL CONSIDERATIONS.

If blood is allowed to flow directly from an artery into a vessel surrounded by a freezing mixture, and containing one-seventh its volume of a saturated solution of sodium sulphate, or a 25 per cent. solution of magnesium sulphate (1 volume to 4 volumes of blood), it will be observed that after some time a sediment, presenting the color of arterial blood, has formed at the bottom, which is covered by a layer of clear, straw-colored fluid—the blood plasma. Upon microscopic examination the sediment will be seen to contain:

(*a*) Numerous homogeneous, non-nucleated, circular, biconcave disks. These measure on an average 7.5 μ in diameter, and are of a faint greenish-yellow color when viewed through the microscope, while *en masse* they present the color of arterial blood—the erythrocytes or red corpuscles of the blood.

(*b*) Roundish or irregularly shaped nucleated cells which are for the most part granular and far less numerous than the red corpuscles, and devoid of coloring matter—the leukocytes, colorless or white corpuscles of the blood.

(*c*) Minute colorless disks, measuring less than one-half the diameter of a red corpuscle—the so-called blood plaques, or blood plates of Bizzozero.

GENERAL CHARACTERISTICS OF THE BLOOD.

Color.—Chemical examination of the blood shows that its color is referable to the presence of an albuminous, iron-containing substance—hemoglobin—in the bodies of the red corpuscles, which is characterized by its great avidity for oxygen, and forms a compound therewith, known as oxyhemoglobin. The relatively larger amount of the latter encountered in the arteries, as compared with the veins, causes the difference in the appearance of arterial and venous blood, the former presenting a bright scarlet-red, the latter a dark-bluish color. A bright cherry-red color is noted in poisoning with carbon monoxide, while a brownish-red or chocolate color is observed in poisoning with potassium chlorate, anilin, hydrocyanic acid, and nitrobenzol. A milky appearance is frequently seen in well-marked leukemia. In chlorosis and hydremic conditions, as would be expected, the blood is pale and watery.

Odor.—The peculiar odor of the blood, which varies in different animals, the *halitus sanguinis* of the ancients, is due to the presence of certain volatile fatty acids, and may be rendered more distinct by the addition of concentrated sulphuric acid.

Specific Gravity.—The specific gravity of the blood in healthy adults varies between 1.058 and 1.062, being higher on an average in men, 1.059, than in women, 1.056, and children—boys 1.052, girls 1.050. Generally speaking, it is proportionate to the amount of hemoglobin and the volume of red corpuscles. It is diminished by fasting, the ingestion of solids and liquids, gentle exercise, pregnancy, etc. It depends, moreover, upon the bloodvessel from which the specimen is taken, being higher in venous than in arterial blood.

Under pathological conditions the specific gravity may vary between 1.025 and 1.083. In nephritis, chlorosis, the anemias in general, and in cachectic conditions (carcinoma of the stomach, etc.) it may diminish to 1.031. In phthisis it is diminished in the third stage (1.040 to 1.042), and in the first stage (1.049) in those patients in whom the onset has been very gradual. In the second stage normal figures are obtained (1.058 to 1.060), corresponding to the relatively high percentage of hemoglobin (90 to 95 per cent.) which is then noted, and which is referable no doubt to a concentration of the blood. An increased specific gravity is met with in febrile diseases (typhoid fever, 1.057 to 1.063), conditions associated with pronounced cyanosis (emphysema, fatty heart, uncompensated valvular disease, 1.054 to 1.068), and obstructive jaundice, 1.062. The highest values have been found in enterogenous cyanosis, 1.067 to 1.083.

Method of Determining the Specific Gravity of the Blood.

Hammerschlag's Method.—A carefully dried cylinder, measuring about 10 cm. in height, is partly filled with a mixture of chloroform (sp. gr. 1.526) and benzol (sp. gr. 0.889), having a specific gravity of 1.050 to 1.060. Into this solution a drop of blood is allowed to fall directly from the finger, pressure being avoided, and care taken that the drop does not come in contact with the walls of the vessel. The drop should not be too large, as otherwise it will separate into droplets, giving rise to inaccurate results. Should the drop sink to the bottom, it is apparent that the specific gravity of the mixture is lower than that of the blood, necessitating the addition of chloroform. This should be added drop by drop while the mixture is thoroughly stirred. If, on the other hand, the drop should tend toward the surface it is best to add an amount of benzol sufficient to cause the blood to sink to the bottom, and then to bring it to the proper degree of suspension by the subsequent addition of chloroform. As soon as the drop remains suspended the mixture is filtered, and its specific gravity ascertained by means of an accurate areometer registered to the fourth decimal. The figure obtained is the specific gravity of the blood. The chloroform-benzol mixture may be kept indefinitely.

With practice, results sufficiently accurate for clinical purposes may thus be obtained with an expenditure of very little time. The examination should in each case be made at the same hour, as the specific gravity undergoes diurnal variations.

Instead of the chloroform-benzol mixture, one of chloroform and olive oil may be employed, as suggested by Van Spanje. It has the advantage of being less volatile than the other. Three parts of chloroform and one of oil give a mixture with a specific gravity of 1.056.

Schmaltz and Peiper's Method.—Where delicate scales are available the method of Schmaltz and Peiper may be employed. A capillary tube, measuring about 12 cm. in length and 1.5 mm. in width, with its ends tapering to a diameter of 0.75 mm., is filled with blood and carefully weighed. The weight of the blood, divided by the weight of an equivalent volume of distilled water, indicates the specific gravity.

As the result of numerous investigations it may now be regarded as an established fact, that with the exception of nephritis, circulatory disturbances, leukemia, posthemorrhagic anemia, and that resulting from inanition, the specific gravity of the blood varies directly with the amount of hemoglobin and the volume of the red corpuscles. A simple method is thus given by means of which hemoglobin estimations can be made in the absence of more expensive instruments. In the following table the specific gravities, as obtained with Hammerschlag's method, and that of Schmaltz and Peiper are given, with the corresponding amounts of hemoglobin:

Specific gravity according to Hammerschlag.	Hemoglobin.	Specific gravity according to Schmaltz and Peiper.	Hemoglobin.
1.033-1.035	25-30 per cent.	1.030	20 per cent.
1.035-1.038	30-35 "	1.035	30 "
1.038-1.040	35-40 "	1.038	35 "
1.040-1.045	40-45 "	1.041	40 "
1.045-1.048	45-55 "	1.0425	45 "
1.048-1.050	55-65 "	1 0455	50 "
1.050-1.053	65-70 "	1.048	55 "
1.053-1.055	70-75 "	1.049	60 "
1.055-1.057	75-85 "	1.051	65 "
1.057-1.060	85-95 "	1.052	70 "
		1.0535	75 "
		1.056	80 "
		1.0575	90 "
		1.059	100 "

LITERATURE.—Schmaltz, Deutsch. Arch. f. klin. Med., vol. xlvii, p. 145; and Deutsch. med. Woch., 1891, No. 16. Stintzing u. Gumprecht, Deutsch. Arch. f. klin. Med., vol. liii, p. 265. Siegl, Prag. med. Woch., 1892, No. 20; and Wien. med. Woch., 1891, No. 33. Hammerschlag, ibid., 1890, p. 1018; and Zeit. f. klin. Med., 1892, vol. xxii, p. 475. Schmaltz, Deutsch. Arch. f. klin. Med., 1890, vol. xlvii, p. 145; and Deutsch. med. Woch., 1891, vol. xvii, p. 555. Appelbaum, Berl. klin. Woch., 1901, vol. xxxix, p. 7.

Reaction.—The reaction of the blood during life, owing to the presence of disodium phosphate and sodium carbonate, is alkaline. The degree of alkalinity in healthy adults, while fasting, corresponds to about 300 to 325 mgrms. of sodium hydrate for 100 c.c. of blood (Löwy). Variations amounting to 75 mgrms. plus or minus are, however, not uncommon and in part due to unavoidable errors of technique (30 mgrms.).

Generally the alkalinity of the blood is lower in women and children than in men, and is influenced by the process of digestion, exercise, etc. At the beginning of digestion, when hydrochloric acid is being freely secreted, the alkalinity of the blood increases; while later on it diminishes. Higher values are usually found during pregnancy than in the non-pregnant state. A decrease is observed following violent muscular exercise and also after the prolonged use of acids, while an increase is brought about by the ingestion of alkalies. An increase in the alkalinity of the blood occurs after a cold bath, and it is interesting to note that this is apparently associated with an increase in the bactericidal power of the blood.

Under pathological conditions the alkalinity may be diminished or increased, as is shown in the table below. Unfortunately we are not able to account for these fluctuations in a satisfactory manner and the data are thus of little value. A marked decrease in diabetes may be viewed as of serious prognostic omen and as indicating acid intoxication. During diabetic coma the reaction owing to the presence of large amounts of beta-oxybutyric acid may actually be acid. The supposition that in gout a diminished alkalinity exists in the intervals between attacks, and that this increases beyond the normal during the attack, has been proved unfounded.

Orlowsky has recently expressed the opinion that the variations in the alkalinity of the blood which have been noted in various diseases and sometimes in one and the same disease, by various investigators working with the older methods, are referable to the varying tonicity of the blood and its varying richness in red corpuscles. Working with blood plasma Orlowsky found a marked diminution of the alkalinity in advanced uremia, in cancerous cachexia, and in severe cases of diabetes, while in other diseases normal values or at most but slight and exceptional variations were observed.

The following table gives some of the results which have been obtained with Löwy's method:

Carcinoma œsophagi	227–643
Carcinoma ventriculi	256–635
Ulcus ventriculi	302–460
Anadeny of the stomach	354–360
Alcoholic gastritis	343–379
Chronic enteritis	212–272
Phthisis pulmonalis	450–468
Bronchitis	239–343
Neurasthenia	225–426
Arteriosclerosis	208–344
Chronic arthritis	368–465
Erysipelas	498
Typhoid fever	270–640
Pneumonia	263–464
Septicemia	443
Leukemia	368–835
Pernicious anemia	429
Diabetes mellitus	362–457
Chronic interstitial nephritis	310–409
Chronic parenchymatous nephritis	312–490
Cirrhosis of the liver	272–345

The alkalinity may be measured according to one of the following methods:

Löwy's Method.—Five c.c. of blood, obtained from one of the superficial veins of the arm (preferably the median cephalic), are allowed to flow into a small flask provided with a long and partially graduated neck, and containing 45 c.c. of a 0.25 per cent. solution of ammonium oxalate. Coagulation is thus prevented and the blood made lake-colored—*i. e.*, the hemoglobin is dissolved from the stroma of the red corpuscles. The mixture is then titrated with a $\frac{1}{25}$ normal solution of tartaric acid, using lacmoid paper, soaked in a concentrated solution of magnesium sulphate, as an indicator.

As a normal solution of tartaric acid contains 75 grams to the liter, a $\frac{1}{25}$ normal solution will contain 3 grams, and 1 c.c. of the $\frac{1}{25}$ normal solution will corrrespond to 0.0016 gram of sodium hydrate.[1]

Supposing that 10 c.c. of the $\frac{1}{25}$ normal solution were necessary to neutralize 5 c.c. of blood, the alkalinity of these 5 c.c. in terms of sodium hydrate would correspond to 0.016 gram, and the alkalinity of 100 c.c. of blood to $0.016 \times 20 = 0.320$ gram—*i. e.*, to 320 mgrms.

Engel's Method.—This is essentially a modification of Löwy's method, and is well adapted for clinical purposes, as the amount of blood required for a single examination can readily be obtained by ordinary puncture.

The blood is measured and rendered lake-colored in a specially constructed pipette (Fig. 1). To this end the blood is drawn to the 0.05 c.c. mark and diluted with *neutral* distilled water, so that the volume of the mixture reaches the 5 c.c. line. After slight agitation the solution is placed in a small beaker and titrated with a $\frac{1}{75}$ normal solution of tartaric acid, from a special burette which accompanies the pipette. This is so constructed that each cubic centimeter is divided into twenty parts. Before and after the addition of every drop of the titrating fluid the reaction of the mixture is tested by placing a drop upon lacmoid paper. The end reaction is reached when the yellow drop of the blood mixture shows a distinct red line along the margin. The result is expressed in terms of milligrams of sodium hydrate per 1 c.c. of blood. Normally about 10 c.c. of the acid solution are employed. The tartaric acid solution contains 1 gram of Merck's crystals (crystallized reagent) to the liter, so that 1 c.c. corresponds to 0.533 mgrm. of sodium hydrate.

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[1] Regarding the standardization of normal solutions the reader is referred to special works on quantitative analysis.

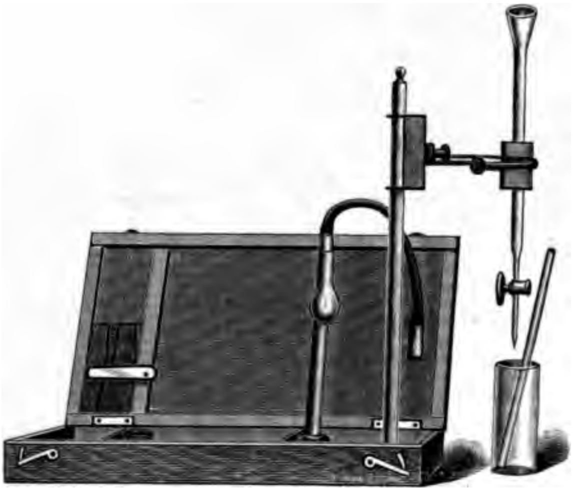

FIG. 1.—Engel's alkalimeter.

Supposing that 0.6 c.c. of the acid solution was required to neutralize the 0.05 c.c. of blood, then 12 c.c. would be necessary for 1 c.c. of blood. As 1 c.c. of the acid solution represents 0.533 mgrm. of sodium hydrate, the alkalinity of 1 c.c. of blood would correspond to 12×0.533—*i. e.*, to 6.396 mgrms.

Dare's Method.—This method is based upon the fact that the characteristic spectrum of oxyhemoglobin disappears at the point of exact neutralization when the blood is titrated with a dilute solution of tartaric acid.

The examination is made with the aid of a special instrument, the *hemo-alkalimeter*, which is pictured in the accompanying illustration (Fig. 2). *B* is a glass stopper through which passes an automatic capillary blood pipette of 20 c.mm. capacity, the exposed end of which is ground to a tapering point. The stopper fits into the tube

A, which has a capacity of 3 c.c. and is graduated in cubic centimeters. The upper end of the tube is blown into a bulb with a minute aperture at *C*. A 2 c.c. dropping tube provided with a short piece of rubber tubing accompanies the instrument.

To neutralize the blood a $\frac{1}{200}$ normal solution of tartaric acid is used, which should contain an amount of alcohol sufficient to prevent the growth of bacteria, but insufficient to precipitate the albumins of the blood. The reagent may be prepared by dissolving 0.075 gram of tartaric acid (Merck's crystals; guaranteed reagent) in a small amount of distilled water, adding 20 c.c. of alcohol (93–94 per cent.), and diluting to 200 c.c. with water.

For the spectroscopic examination a Browning instrument (Fig. 3) will suffice.

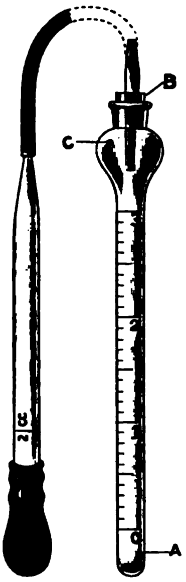

Fig. 2.—Dare's hemo-alkalimeter.

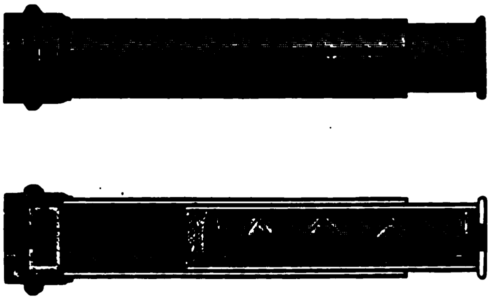

Fig. 3.—Browning's spectroscope. (Zeiss.)

Method.—A drop of blood is obtained from the finger-tip or the lobe of the ear in the usual manner. The blood pipette is filled *in situ* by capillary attraction, holding the instrument horizontally to the drop of blood as it emerges from the wound. With an ordinary medicine dropper filled with distilled water the blood is washed into the bottom of the tube, connecting the dropper with the pipette by means of a short piece of rubber tubing. Blood and water should just reach the zero mark, and are intimately mixed by closing the aperture in the bulb with the finger and inverting the tube several times. The *reagent pipette* is then filled with the tartaric acid solution and the rubber tubing slipped over the outer end of the blood pipette; by compressing the rubber bulb the acid solution is forced through the pipette into the test-tube, the aperture in the glass bulb being closed before the pressure is relaxed. Having done this the tube is inverted several times while still attached to the reagent pipette, taking care that this is held vertically so that the acid solution does not get into the rubber bulb. The tube is clamped in front of the spectroscope and examined for the two bands of oxyhemoglobin. (See Fig. 6.) So long as these are visible more of the acid is added, inverting the tube after each addition; as the bands become fainter one drop at a time is allowed to enter. At first this is rather tedious, but after several examinations have been made it will be found unnecessary to apply the spectroscope so frequently to determine the point of neutralization, as the eye rapidly learns to recognize this by the characteristic change of color of the blood mixture. The observation is at an end when the oxyhemoglobin bands have just disappeared.

The examination is made with artificial light, keeping the distance from the light constant.

Dare suggests that for sake of convenience the results be expressed in terms of the number of cubic centimeters of the tartaric acid solution instead of in mgrms. of sodium hydrate, as has been customary. The corresponding values are given in the table below, and have reference to 100 c.c. of blood. His normal values range between 266 and 292.

C.c. of reagent:	Equivalent in terms of mgrms. of NaOH per 100 c.c. of blood.
2.6	345.0
2.4	319.0
2.2	292.0
2.0	266.0
1.8	239.0
1.6	212.0
1.4	176.0
1.2	169.0
1.0	133.0
0.8	96.0
0.6	79.0
0.4	53.0
0.2	26.6

Dare has ascertained with his method that there is a more or less constant relationship between the alkalinity of the blood and the color index, and he suggests that this may be the reason why the results obtained by different investigators differ so widely, as at different stages of the disease the color index may change.

The method is quite convenient and merits the careful attention of all laboratory workers.

LITERATURE.—v. Jaksch, Zeit. f. klin. Med., 1887, vol. xiii, p. 350. A. Löwy. Arch. f. d. gesammte Physiol., 1894, vol. lviii, p. 462. Löwy u. Richter, Deutsch. med. Woch., 1895, vol. xx, p. 526. Peiper, Arch. f. path. Anat., 1889, vol. cxvi, p. 337. Rumpf, Centralbl. f. inn. Med., 1891, vol. xii, p. 447. Kraus, Arch. f. exp. Path. u. Pharmakol., vol. xxvi. Engel, Berlin. klin. Woch., 1898, p. 308. Brandenburg, Zeit. f. klin. Med., vol. xxxvi, p. 267. Orlowsky, Wratch, 1902, vol. xxii, pp. 1190 and 1222. A. Dare, Phila. Med. Jour., Jan. 17, 1903; and Johns Hopkins Hospital Bull., July, 1903.

CHEMICAL EXAMINATION OF THE BLOOD.

Chemical Composition of the Blood.—A general idea of the chemical composition of the blood may be formed from the accompanying table of C. Schmidt, calculated for 1000 parts:

	Man.	Woman.
Corpuscles	513.00[1]	369.20
Water	349.70	272.60
Hemoglobin and globulins	159.60	120.10
Mineral salts	3.70	3.55
Plasma	486.90	603.80
Water	439.00	552.00
Fibrin	3.90	1.91
Albumins and extractives	39.90	44.79
Mineral salts	4.14	5.07

Blood plasma differs from blood serum in the presence of fibrinogen in the former and its absence in the latter. The substance is used up during coagulation, fibrin and a small amount of fibrinoglobulin resulting.

The albumins which are common to both plasma and serum are serum albumin and serum globulin. Of these the globulin is the larger fraction (3.84 as compared with 2.6 per cent., in horses' blood).

From the following table it will be seen that a marked difference exists in the nature of the mineral ingredients between serum and the red corpuscles, the latter being relatively rich in potassium salts and phosphorus, and poor in sodium salts and chlorine. The figures have reference to 1000 parts of blood

	Man.		Woman.	
	Red corpuscles.	Serum.	Red corpuscles.	Serum.
K_2O	1.586	0.153	1.412	0.200
Na_2O	0.241	1.661	0.648	1.916
CaO	. .	. .	. .	. .
MgO	. .	. .	. .	. .
Fe_2O_5	. .	. .	. .	. .
Cl	0.898	1.722	0.362	1.440
P_2O_5	0.695	0.071	0.643	2.202

It is noteworthy that the amount of sodium chloride in the serum, 6 to 7 pro mille, remains fairly constant no matter whether large amounts are ingested or none at all is given. The term "isotonic" has been applied to a salt solution which is just strong enough to prevent the solvent action of the water upon the hemoglobin of the red corpuscles. In the case of the serum we meet with a condition of hyperisotonia—*i. e.*, an amount of salt in excess of that actually required in order to prevent the destruction of the red corpuscles.

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[1] This figure is too high; in man it varies between 420 and 470 for 1000 parts of blood.

Fat occurs in amounts varying from 1 to 7 pro mille in fasting animals, while following the ingestion of a meal rich in fats as much as 12.5 pro mille have been encountered.

Soaps, cholesterin, and lecithin have likewise been found.

Glucose is a normal constituent of the plasma, amounting to from 1 to 1.5 pro mille in man. While it is possible to increase this amount to a certain degree by the ingestion of large quantities of sugar, this appears in the urine, according to Claude Bernard, as soon as 3 pro mille have been exceeded. In addition to glucose, another reducing substance has been found in the blood, which differs from the former in not being fermentable. According to the researches of P. Mayer,[1] this is in all probability a glucuronic acid compound. Whether jecorin also occurs in the blood is doubtful.

Among the extractives which have been found there may be mentioned urea, uric acid, kreatin, carbamic acid, sarcolactic acid, glycogen, hippuric acid, and under pathological conditions xanthin, hypoxanthin, paraxanthin, adenin, guanin, leucin, tyrosin, lactic acid, cellulose, β-oxybutyric acid, acetone, and biliary constituents.

It has been pointed out that the color of the blood is referable to the presence of hemoglobin in the red corpuscles, and that it varies from a bright scarlet-red in the arteries to a dark bluish-red in the veins, the exact shade depending upon the amount of oxygen present in combination with hemoglobin as oxyhemoglobin. Upon chemical examination two other gases may be demonstrated under physiological conditions, viz., carbon dioxide and nitrogen. Of these, the latter appears to play no part in the body economy, and the amount present merely corresponds to that which would be absorbed by an equal volume of distilled water, viz., 1.8 vol. per cent., calculated at 0° C. and 760 Hgmm. pressure.

The amount of oxygen and carbon dioxide, on the other hand, undergoes considerable variation, depending upon the particular bloodvessel from which the specimen is taken—*i. e.*, whether this be an artery or a vein, and, furthermore, upon the velocity of the blood current, the temperature of the body, rest, exercise, etc.

The relation existing between the amounts of these gases in arteries and veins may be seen from the following table:

	Arterial blood.	Venous blood.
Oxygen	21.6 per cent.	6.8 per cent.
Carbon dioxide . . .	40.3 "	48.0 "
Nitrogen	1.8 "	1.8 "

Oxygen, as already pointed out, occurs principally in chemical combination with hemoglobin (oxyhemoglobin), only 0.26 per cent. being present in solution in the plasma.

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[1] Zeit. f. physiol. Chem., vol. xxxii, p. 518.

Of the carbon dioxide which may be obtained from the blood, only one-tenth is held in solution. One-third is found in the red corpuscles, in the form of a loose compound with the alkalies of the corpuscles, and possibly also in combination with hemoglobin. The remaining portion is held in chemical combination by the alkalies of the plasma and albuminous bodies.

Coagulation.—If blood is allowed to flow into a vessel and set aside, it will be observed at the expiration of a few minutes that the entire mass has become transformed into a semisolid, gelatinous material, which is spoken of as the blood clot or the *placenta sanguinis*. Still later it will be seen that a small amount of straw-colored fluid appears on top of the clot, which gradually increases in amount, while the clot itself undergoes shrinkage, until finally it floats, greatly diminished in size, in the surrounding fluid. The straw-colored fluid which has thus been obtained during the process of coagulation is spoken of as the *blood serum*.

If a bit of the clot is examined microscopically, it will be seen to consist of a more or less dense network of fibers, the meshes of which are filled with blood corpuscles, which may be washed out, leaving the fibrous network, fibrin, behind.

Chemically speaking, fibrin belongs to the class of the so-called coagulated albumins; it does not occur in the circulating blood, but is formed only during the process of coagulation.

Under normal conditions blood clots in from two to six minutes after being shed, while in disease, notably in hemophilia, coagulation may be greatly retarded or does not occur at all, so that fatal hemorrhage may follow the infliction of trifling wounds. A tendency to hemorrhage is also observed in scurvy, purpura, in some infectious diseases, such as typhoid fever and yellow fever, in poisoning with phosphorus,[1] etc. Sicard[2] has pointed out that in purpura primary coagulation occurs as with normal blood, but that subsequent retraction of the clot and exudation of serum take place to only a very limited extent. Normal serum when added to fluids, such as hydrocele fluid, which are not spontaneously coagulable, in the proportion of 1 to 80, induces coagulation in from four to six hours.

The serum of purpuric patients, on the other hand, is either entirely devoid of this property or possesses it to only a very slight degree. The addition of a trace of calcium chloride, however, causes such serum to behave very much like normal serum. Sicard hence suggests that in certain cases of purpura the fibrin ferment or its pro-enzyme is not present in sufficient quantity to cause more than a primary coagulation.

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[1] Schmidt, Pflüger's Archiv, vol. xi, pp. 291 and 515. Bojanus, Inaug Diss., Dorpat, 1881.

[2] Compt.-rend. Soc. biolog., vol. li, p. 579.

Wright's Coagulometer.—Wright's coagulometer may be conveniently employed to determine the rapidity of coagulation. The instrument is shown in the accompanying illustration (Fig 4). The essential parts are a tin water can, a thermometer registered to about 50° C., and a set of glass tubes measuring about 10 cm. in length with a lumen of 0.25 mm. and marked at 5 cm. When the instrument is to be used, the can is filled with water having a temperature about that of the body. The tubes are covered at their distal ends with little rubber caps and placed in their respective positions in the water bath, where they remain until they have acquired a similar temperature.

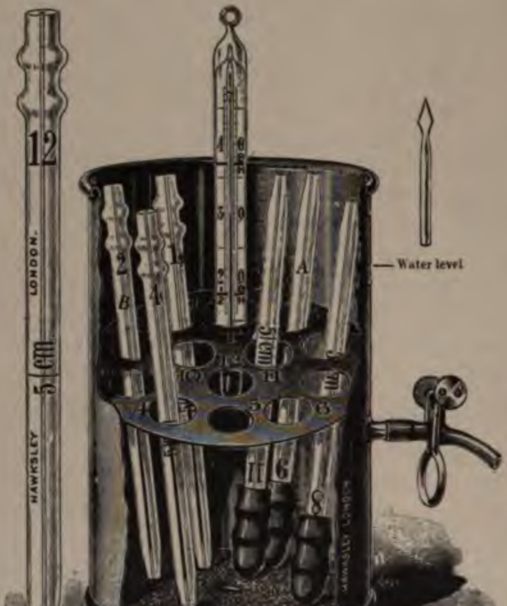

Fig. 4.—Wright's coagulometer.

They are then successively filled about one-half by aspiration from a drop of blood obtained from the finger or the lobe of the ear and replaced (properly numbered) tips down into the water in their proper positions. Careful note is kept of the exact time when they are filled. When a series of six or eight tubes has been filled, tube No. 1 is withdrawn from the water, and is held over a piece of white filter or blotting paper. The condition of the coagulation is then tested by blowing into the tube, the time of testing and the result being noted on the record.

1. If the contents cannot be blown out, an entry is made on the record that coagulation is "complete."
2. If the contents can be blown out, but if shreds of fibrin are found adhering to the inside of the tube or to the filter paper, coagulation is recorded as "incomplete."
3. If, lastly, the contents can be blown out cleanly, and if no trace of fibrin is seen on the filter paper, a note is made that coagulation has not yet begun.

In the first case the second tube is immediately taken in hand and is tested in the same manner as the first. If this is found clotted the next in series is tested, and so on, until a tube is found in which coagulation is still incomplete.

In the second case, i. e., in the case where coagulation is found to be still incomplete, a slightly longer interval—reckoning from the time at which the second tube was filled in—is allowed to elapse before the tube next in series is tested.

Lastly, if it is found that coagulation has not yet begun, an interval of one minute or more is allowed to elapse before testing the tube next in series.

When the condition of the blood in a coagulation tube has been once tested, the tube in question must be put aside. Even if it has not all been blown out of the tube, its rate of coagulation will have been disturbed by the movement.

Under normal conditions the coagulation time with these tubes will be found to vary between three and five minutes. The temperature of the water in the can should be kept uniform during the examination by adding hot water if necessary.

The tubes are cleansed by removing the clots with a fine wire; they are then washed with water, with alcohol, and finally with ether.

THE BLOOD PIGMENTS.

Hemoglobin and Oxyhemoglobin.—Hemoglobin is a proteid which is composed of an albuminous radicle, *globin*, and a non-albuminous pigment radicle, *hemochromogen*. Upon the presence of the latter depends the readiness with which hemoglobin forms compounds with certain gases, such as oxygen, carbon monoxide, carbon dioxide, nitric oxide, and cyanogen. Hemochromogen in combination with oxygen is known as hematin. Oxyhemoglobin thus differs from hemoglobin merely in the fact that the pigment radicle is present in combination with oxygen.

By itself hemoglobin is largely found in the blood of asphyxia. Under ordinary conditions it is principally present as oxyhemoglobin; in arterial blood this preponderates, while in venous blood a mixture of both is found.

On spectroscopic examination hemoglobin in suitable dilution shows a single band of absorption between *D* and *E*, extending slightly beyond *D* to the left (Fig. 5).

Oxyhemoglobin shows two bands of absorption between *D* and *E*. One band, *a*, which is not so wide as the second, *B*, but darker and more sharply defined, borders on *D;* the second, which is wider but less sharply defined, lies at *E* (Fig 6). This spectrum can be readily transformed into that of hemoglobin by the addition of a reducing agent, such as ammoniacal solution of ferrous tartrate (Stokes' fluid), ammonium sulphide, or cuprous salts.

Under normal conditions the amount of hemoglobin is fairly constant, but varies somewhat in different countries with the habits of the people, the character of the diet, etc. In Germany, as the result of 61 estimations, Leichtenstern found 14.16 per cent. by weight as the average in healthy men, and 13.10 per cent. in women.

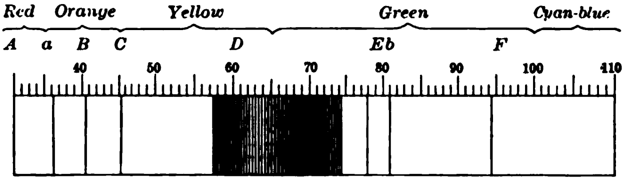

FIG. 5.—Spectrum of reduced hemoglobin. (v. Jaksch.)

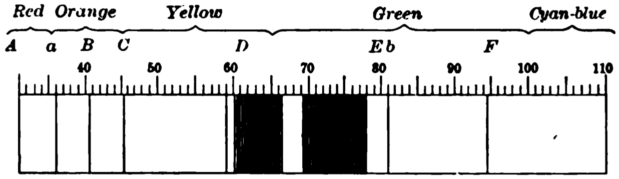

FIG. 6.—Spectrum of oxyhemoglobin. (v. Jaksch.)

Clinically we express the amount of hemoglobin by relative figures as compared with the average normal percentage by weight; on this basis the scale of the various hemoglobinometers is constructed. On these instruments the figure 100 represents the average normal value; this, however, varies somewhat with the various forms of hemoglobinometers according to the average percentage by weight which has been taken as a standard in establishing the 100 mark. With the Gowers instrument Strauss and Rohnstein obtained figures varying between 85 and 125 as normal values; this would furnish an average of 105. Schaumann and v. Willebrandt give 88 as the average normal. With the v. Fleischl instrument I rarely find higher values than 90 per cent. in inhabitants of large cities, but with the Dare apparatus the average results more nearly approach the 100 mark.

In children the average values are somewhat lower than in the adult. Stierlin gives 79.7 per cent. for boys and 82.1 for girls. Borchmann's values are even lower, viz., 55 and 80; Gundobin gives 70 and 95.

The ingestion of large amounts of water does not cause a dilution of the blood and hence a diminution of the amount of hemoglobin; but relatively higher values are found upon the withdrawal of liquids, owing to a concentration of the blood as a whole. Fat persons show smaller values than correspond to their age.

Pathological Variations.—Abnormally high values, *hyperchromemia*, viz., 120 to 150 per cent., occur in cases of chronic enterogenous cyanosis, and may also be observed in congenital heart disease. The hyperchromemia in these cases is associated with polyglobulism.

A pathological decrease is spoken of as *oligochromemia*, and is observed in all forms of anemia from whatever cause.

The lowest values are found in chlorosis, in which the oligochromemia far exceeds the *oligocythemia*, viz., the diminution in the number of the red cells. In an analysis of 94 cases I found an average of 42.5 per cent.; the lowest value was 17.5 (Fleischl). There are instances on record in which the reading was still lower.

Very low figures are seen in splenic anemia, and it is rare, excepting in chlorosis, to find such a low grade of chromemia associated with a blood count which is normal or may indeed be above normal. The average of 13 estimations given by Osler was 47 per cent.

In pernicious anemia the oligocythemia exceeds the oligochromemia. The loss of hemoglobin is, however, also quite marked and may be as great as in the most extreme cases of chlorosis. In the series of 23 cases collected by Strauss and Rohnstein the average value was 25 per cent. (Gowers); in 9 cases it was lower than 20 per cent. A. Meyer reports a bothriocephalus case with only 10 per cent.

In the early stages of leukemia the loss of hemoglobin is often not especially marked; later the anemia may become quite intense, but the oligochromemia is not necessarily of high grade even in well-developed cases. Ehrlich cites cases in which the Gowers instrument gave readings of from 60 to 70 per cent. On the other hand, there are cases in which the oligochromemia is an early feature of the disease, and in one instance of this kind I obtained a reading of only 27 per cent. Cases of this order have been described as *leukanemia.* The blood picture is essentially a composite of leukemia and pernicious anemia.

While in the course of typhoid fever the amount of hemoglobin is always reduced (Osler), and usually to a greater extent than the number of the red corpuscles, the most severe grades of anemia may be encountered during convalescence, when the amount of hemoglobin may fall to 20 per cent.

In the early stages of carcinoma of the stomach the cachexia is not well pronounced. Schüle states that in his analysis of 198 cases it occurred in only 30 per cent. Later the loss of hemoglobin is quite marked; the values may indeed approach those seen in chlorosis and pernicious anemia.

An intense grade of anemia is seen in generalized septicemia, and, as Ewing remarks, no form of the acute disease appears to act more violently than does puerperal or uterine sepsis. A diminution in the amount of hemoglobin to 20 per cent. is here not uncommon. In the chronic cases also a high grade of oligochromemia is a constant feature. In a case of lumbar abscess of six months' duration I found 21 per cent. of hemoglobin, with 1,025,000 red cells. The hemoglobin in all these cases diminishes more rapidly than the number of the red cells.

In pulmonary tuberculosis a diminution in the amount of hemoglobin is seen essentially in the third stage of the disease (40 to 45 per cent.), while previously fairly normal values are obtained (90 to 95 per cent.). It is to be noted, however, that a certain grade of anemia (69 per cent.) is quite commonly observed, even in the first stage, in those cases in which the disease has been of very gradual onset, viz., in patients who often have suffered from tuberculous affections (scrofula) since childhood. In the third stage the anemia is well marked (40 to 50 per cent.).

A notable diminution in the amount of hemoglobin is observed in chronic nephritis, chronic enteritis, in chronic lead and mercurial poisoning, in syphilis, etc.

In syphilis the anemia develops at a time when the entire organism has been thoroughly infected. The lowest hemoglobin values are reached just before or coincidently with the appearance of the rash. In the secondary stage the degree of oligochromemia, *cœteris paribus*, may be regarded as a fair index of the severity of the infection. In untreated cases the hemoglobin remains low for several days or even for weeks. A gradual rise then occurs which is associated with beginning involution of the exanthem. In uncomplicated cases normal values may subsequently be reached even without treatment; a fall again occurs with relapses. Similar changes are observed in the tertiary stage. Especially interesting are the observations of Justus on the blood changes which occur in the course of mercurial treatment; Justus ascertained that a rapid and material diminution of the hemoglobin (10 to 20 per cent.) occurs when a large (medicinal) amount of mercury is introduced at one time into the body of the infected individual. This decrease is only observed in the blood of patients with florid syphilis; it is specific and does not occur in healthy nor in otherwise diseased individuals. The reaction is demonstrable in every form of syphilitic infection (secondary, tertiary, and hereditary) as soon as the more distant lymph glands begin to swell. It disappears, or is at least no longer demonstrable, with beginning involution of the symptoms.

The practical value of this *syphilitic blood test* has not yet been definitely established. While some observers have expressed themselves against its value, it must be recognized that in discussing his adversaries' criticisms Justus seems to have maintained the upper hand.

During anesthesia by ether the amount of hemoglobin is always absolutely reduced. In some instances there is an apparent increase, but this is never proportionate to the rise in the number of the red cells which is simultaneously observed (Da Costa, Kalteyer). Owing to the hemocytolysis which thus undoubtedly takes place a very low percentage of hemoglobin should be regarded as a counterindication to general anesthesia. A lower value than 50 per cent. is now regarded by many as a dangerous figure.

For the estimation of hemoglobin see p. 147.

LITERATURE.—Strauss u. Rohnstein, Die Blutzusammensetzung b. d. verschiedenen Anæmien, Hirschwald, Berlin, 1901. Appelbaum, Berl. klin. Woch., 1901, vol. xxxix, p. 7. Quincke, "Zur Pathologie d. Blutes," Deutsch. Arch. f. klin. Med., vols. xxv and xxvii. Leichtenstern, Unters. über d. Hemoglobingehalt d. Blutes im gesunden u. kranken Zustande, Leipzig, 1878. W. Osler, "On Splenic Anemia," Am. Jour. Med. Sci., 1902, vol. cxxiv, p. 763. Justus, Virchow's Archiv, vol. cxl, p. 1; and Deutsch. Arch. f. klin. Med., 1902, vol. lxxv, p. 1.

Hemoglobinemia.—The term hemoglobinemia has been applied to a condition in which the hemoglobin is dissolved out from the red corpuscles, and, appearing in the plasma as such, leads at first to a very decided choluria and in extreme cases to hemoglobinuria.

Various poisons, such as potassium chlorate, carbolic acid, pyrogallic acid, naphtol, arsenic, antimony, hydrochloric acid, sulphuric acid, antifebrin, antipyrin, phenacetin, sulphonal, tincture of iodine, when given hypodermically, or even internally in sufficiently large doses, will call forth a hemoglobinemia which is followed by hemoglobinuria.

Fresh morels also contain a poison which is capable of producing an intense hemoglobinuria, and which may be extracted with hot water.

In acute and chronic infectious diseases of a severe type, such as scarlatina, typhoid fever, intermittent fever, icterus gravis, syphilis, as also in diseases depending upon a hemorrhagic diathesis, such as variola hemorrhagica, scurvy, as also following insolation, extensive burns, and frostbite, hemoglobinemia, leading to hemoglobinuria, is not infrequently observed. The same has been noted in splenic anemia and in Raynaud's disease. In syphilis a moderate grade of hemoglobinemia can be demonstrated by spectroscopic examination of the serum within two or three minutes following an intravenous injection of mercuric chloride in medicinal doses. (See also Justus' test.)

An epidemic hemoglobinuria of the newly born and a paroxysmal or intermittent hemoglobinuria, both of unknown origin, have likewise been described.

Hemoglobinemia also follows the infusion of blood of animals of one species into the circulation of animals of a different species.

In some cases, and particularly in those following poisoning with chlorates, etc., the hemoglobinemia ultimately leads to a well-pronounced methemoglobinemia (see below).

A hemoglobinemia, aside from the urinary examination, may be readily recognized by a spectroscopic examination of the serum, when the two bands of absorption of oxyhemoglobin will be observed.

A very simple method which may be employed for the same purpose is the following: One-half to 1 c.c. of blood is collected in a small glass tube, drawn out and sealed at one end. This amount can be readily obtained by puncturing the ear and milking out the blood, which is transferred to the tube by means of a little pipette. After the blood has clotted, the clot is separated from the walls by means of a wire or a glass rod and the corpuscles packed down by centrifugation. With normal serum the supernatant fluid presents a straw-yellow color, while in hemoglobinemia it is colored a more or less intense red.

If the supernatant fluid is withdrawn, diluted with a little water, and heated to 70 to 80° C., the coagulum in the presence of hemoglobin will present a brownish color.

Literature.—Ponfick, Verhandl. d. Cong. f. inn. Med., 1883, vol. ii, p. 205. Stadelmann, Arch. f. exp. Path. u. Pharmakol., 1882, vol. xv, p. 337, and 1884, vol. xvi, pp. 118 and 221. Afanassiew, Zeit. f. klin. Med., 1883, vol. vi, p. 281. v. Jaksch, Verhandl. d. Cong. f. inn. Med., 1891, vol. x, p. 353.

Carbon Monoxide Hemoglobin.—In cases of coal-gas poisoning the blood, both of arteries and veins, presents a bright cherry-red color, owing to the presence of carbon monoxide hemoglobin.

Such blood, when properly diluted, like oxyhemoglobin, shows two bands of absorption between *D* and *E* (Fig. 7), which are nearer the violet end of the spectrum, however, and may readily be distinguished from those referable to oxyhemoglobin by the addition of a reducing agent. This will not affect the spectrum of carbon monoxide hemoglobin, while that of oxyhemoglobin is transformed into the spectrum of reduced hemoglobin.

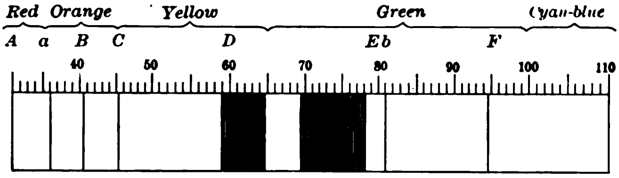

Fig. 7.—Spectrum of carbon monoxide hemoglobin. (v. Jaksch.)

For medico-legal purposes a number of additional tests have been devised, among which that suggested by Hoppe-Seyler is one of the simplest and at the same time most reliable. The blood is treated with double its volume of a solution of sodium hydrate (sp. gr. 1.3). Normal blood is thus changed into a dirty-brownish mass, which exhibits a trace of green when spread upon a porcelain plate, while carbon monoxide blood yields a beautiful red under the same conditions.

Nitric Oxide Hemoglobin.—The blood in cases of poisoning with nitric oxide, owing to the presence of nitric oxide hemoglobin, yields a spectrum which is similar to that of carbon monoxide hemoglobin; the bands, however, are less sharply defined and paler than those of the latter, and, like these, do not disappear on the addition of a reducing substance.

Sulphohemoglobin (Methemoglobin Sulphide).—In cases of poisoning with hydrogen sulphide no definite changes can be discovered in the blood upon spectroscopic examination, although Hoppe-Seyler has shown that hemoglobin may enter into combination with this gas. It is stated, however, that in such cases the blood becomes dark and of a dull-greenish tint, and that the distinction between arterial and venous blood is lost.

A remarkable instance of sulphohemoglobinemia has been described by v. d. Berg,[1] in a case of autotoxic enterogenous cyanosis. In this case an organism producing hydrogen sulphide was isolated from the stools. When grown in a solution of normal oxyhemoglobin sulphohemoglobin resulted.

Carbon Dioxide Hemoglobin.—With carbon dioxide, as mentioned above, hemoglobin is also thought to enter into combination, the spectrum being similar to that of reduced hemoglobin. The latter, in fact, is formed artificially when carbon dioxide is passed through a solution of oxyhemoglobin. If this process is carried farther, the hemoglobin is decomposed and globin is thrown down; an absorption band is then obtained which is similar to that resulting when hemoglobin is decomposed with acids (see below), and is no doubt referable to the presence of free hemochromogen.

Of the blood changes occurring in cases of poisoning with *hydrocyanic acid* and *acetylene* but little is known, and the reader is referred to works on toxicology for their consideration.

Hematin.—If oxyhemoglobin in aqueous solution is heated to a temperature of from 60° to 70° C., it is decomposed into globin and hematin. The same result is reached by treating the aqueous solution with acids, alkalies, or the salts of various heavy metals.

Hematin is an amorphous, blackish-brown, or bluish-black substance which is frequently encountered in old transudates, in the stools after hemorrhages, and after meals consisting largely of red meats. It is said to occur in the urine in cases of poisoning with arsenic, and in the blood of animals poisoned with nitrobenzol its presence can likewise be demonstrated with the spectroscope.

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[1] Arch. f. klin. Med., 1905, vol. lxxxiii, p. 86.

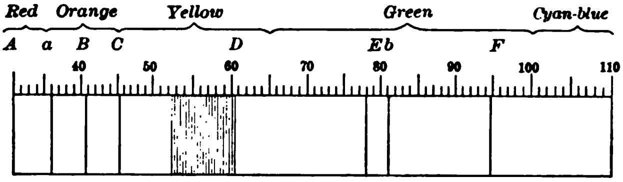


FIG. 8.—Spectrum of hematin in alkaline solution. (v. Jaksch.)

In acid solution it shows a well-defined spectral band between *C* and *D* (Fig. 10). Between *D* and *F* a second band is seen, which is much wider but less sharply defined than the first, and may be resolved into two bands by dilution, one between *b* and *F*, near *F*, and another between *D* and *E*, near *E*; a faint fourth band may also be seen between *D* and *E*, near *D*. As a rule only the two bands between *D* and *F* are visible.

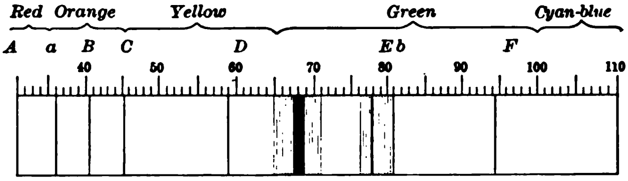


FIG. 9.—Spectrum of reduced hematin. (v. Jaksch.)

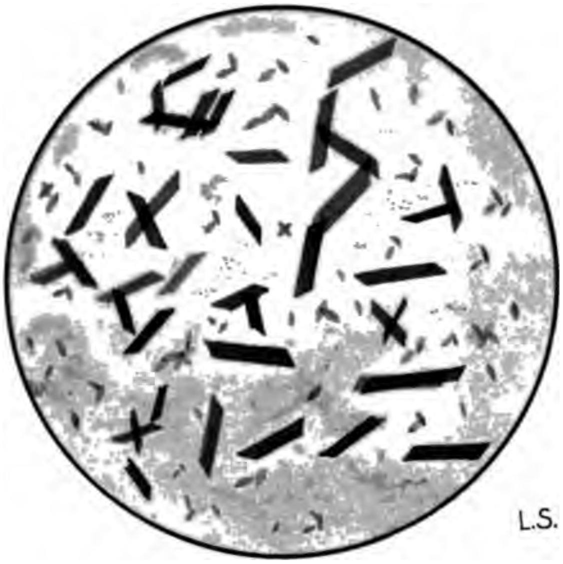
In alkaline solutions it shows but one broad band, the greater portion of which lies between *C* and *D*, extending slightly beyond *D* (Fig. 7).

If an alkaline solution of hematin is treated with a reducing substance, reduced hematin (hemochromogen) results, which gives rise to two absorption bands between *D* and *E* (Fig. 9).

Hemin.—Hematin readily combines with one molecule of hydrochloric acid to form hemin. This substance crystallizes in light-brown or dark-brown rhombic plates or columns, which are quite characteristic (Plate I). They bear the name of their discoverer, Teichmann. The size of these crystals varies with the manner in which they are produced, the largest specimens being met with when the glacial acetic acid (see below) is allowed to evaporate as slowly as possible. Specimens measuring from 15 μ to 18 μ in length may then be seen. Smaller crystals will be present at the same time, occurring either singly or in the form of stars, rosettes, and crosses.

As these crystals may be obtained from mere traces of blood, their

PLATE I.



Hemin Crystals.

formation must be regarded as conclusive evidence in medico-legal examinations. Lewin and Rosenstein have pointed out, however, that under certain conditions a negative result may be reached, even if the coloring matter is derived from the blood. This is the case especially when the hemoglobin has been transformed into hemochromogen or hematoporphyrin, or when substances have been mixed with the blood which are either capable of altering its general composition or which, through their mere presence, interfere with the reaction. Such substances are certain salts of iron (rust), lead, mercury, and silver; further, lime, animal charcoal, and sand, when intimately mixed with the blood. In medico-legal cases a spectroscopic examination should hence be made whenever the hemin reaction is not obtained.

METHOD.—A small drop of normal salt solution is carefully evaporated on a slide, when a few particles of the suspected material, powdered or teased as finely as possible, are placed on the delicate layer of crystallized salt. Glacial acetic acid is now added drop by drop and the specimen carefully heated (three-quarters to one minute) until bubbles begin to form. While evaporation is being continued glacial acetic acid is further added until a light-brown tint appears. As soon as this point is reached, the last traces of the acid are allowed to evaporate, the specimen being held at a greater distance from the flame. A drop of glycerin is then added and the preparation covered with a cover-glass. The examination is made with a one-fifth or a one-sixth objective. Attention is especially directed to brownish streaks or specks, which, in the presence of blood, can usually be made out with the naked eye.

Methemoglobin.—Methemoglobin is a pigment closely related to oxyhemoglobin, and is frequently encountered in hemorrhagic transudates, cystic fluids, and in the urine in cases of hematuria and hemoglobinuria. In the circulating blood methemoglobin is found after

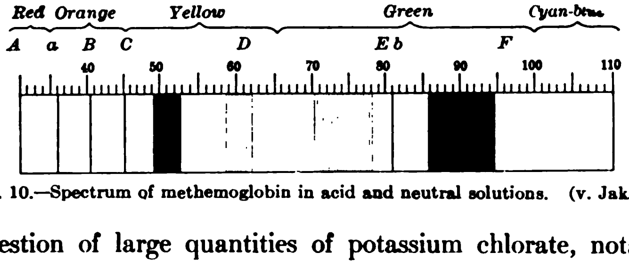


FIG. 10.—Spectrum of methemoglobin in acid and neutral solutions. (v. Jaksch.)

the ingestion of large quantities of potassium chlorate, notably in children, as also after the inhalation of nitrate of amyl, the use of kairin, thallin, hydrochinon, pyrocatechin, iodine, bromine, turpentine, ether, perosmic acid, permanganate of potassium, and antifebrin (see Hemoglobinemia). Most remarkable is the occurrence of methemoglobinemia in cases of so-called autotoxic enterogenous cyanosis, as reported by Stokvis and v. d. Berg. In one case the latter found sulphohemoglobin in the place of methemoglobin.

The spectrum of an aqueous or slightly acidified solution of methemoglobin (Fig. 10) closely resembles that of an acid solution of hematin, but differs from this in the ease with which it is transformed into that of hemoglobin when an alkali and a reducing substance are added. The spectrum of hematin under the same conditions is transformed into that of an alkaline solution of hemochromogen. In alkaline solutions, on the other hand, two bands of absorption are observed, which are similar to those of oxyhemoglobin, but differ from these in the fact that the band nearer *E*, *b*, is more pronounced than the one at *D*, *a*. A third, but very faint, band may further be observed between *C* and *D*, near *D*.

Hematoidin.—Small amorphous particles of an orange or ruby-red color, or crystals belonging to the rhombic system, occurring either singly or in groups, are frequently met with in the sputum, the urine, and the feces, as well as in old extravasations of blood. They were discovered by Virchow, who applied the term hematoidin to this particular pigment, the hemic origin of which is undoubted. It is supposedly identical with bilirubin.

Hematoporphyrin.—Hematoporphyrin is likewise a derivative of hematin, and, according to Nencki and Sieber, isomeric with bilirubin. In dilute solution with sodium carbonate it shows four bands of absorption, one between *C* and *D*; a second one, broader than the first, about *D*, especially marked between *D* and *E*; a third one, not so broad and less sharply defined, between *D* and *E*, and a fourth one, broad and dark, between *b* and *F* (Fig. 11).

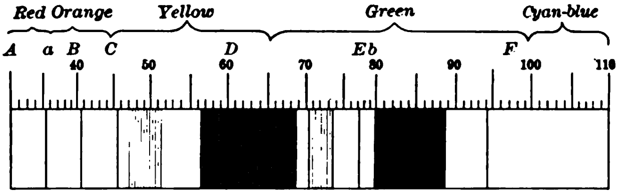


FIG. 11.—Spectrum of hematoporphyrin in alkaline solution.

The clinical significance of this body, which also appears in the urine, as well as the causes which give rise to its formation, are unknown (see Hematoporphyrinuria). It has been found postmortem in the blood, in a case of sulphonal poisoning, by Taylor and Sailer.[1]

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[1] A. E. Taylor and J. Sailer, Contrib. from the William Pepper Laboratory, Phila., 1900, p. 120.

THE PROTEINS OF THE BLOOD.

In considering the proteins of the blood from a clinical point of view, it is necessary to distinguish between an increase and a diminution in their normal amount, constituting the conditions of *hyperalbuminosis* and *hypalbuminosis*, respectively. As may be expected, the former is met with whenever water is more rapidly withdrawn from the system than it can be supplied, and is hence observed in cases of cholera, acute diarrhea, following the use of purgatives, etc. This increase in the amount of proteins is only a relative increase, however. The occurrence of an absolute increase has not been satisfactorily demonstrated. An absolute hypalbuminosis, on the other hand, is observed following a direct loss of proteins from the blood, as in hemorrhage, dysentery, albuminuria of high degree, the formation of large collections of pus, etc. This is generally associated with a relative increase in the amount of water—*i. e.*, a hydremia—which is particularly noticeable after hemorrhages, and referable to a diminished secretion and excretion of water, as well as to a direct absorption from the tissues. Hypalbuminosis has also been observed in pernicious anemia, and is dependent partly upon a diminution in the amount of the albumins of the serum and partly upon a decrease in the weight of the corpuscular solids. The amount of serum-albumin is about normal, while the globulins are much diminished.[1]

The term *hyperinosis* has been applied to a condition in which the amount of fibrin (normally 0.349 to 0.425 per cent.) is increased. This is said to occur in various inflammatory diseases, such as pneumonia, pleurisy, scarlatina, acute articular rheumatism, and erysipelas, while a diminished amount of fibrin, *hypinosis*, or normal values are seen in malaria, nephritis, pyemia, pernicious anemia, typhoid fever, and leukemia (both lymphoid and myeloid).

In order to determine the amount of fibrin, 30 to 40 c.c. of blood, obtained by means of cupping glasses or venesection, are placed in a previously weighed beaker, fitted with an India-rubber cap, through the centre of which passes a piece of whalebone, firmly fixed. The blood is defibrinated by beating with the whalebone, when the beaker with its contents is weighed, the difference indicating the weight of the blood. The beaker is then filled with water and the mixture again beaten. The fibrin is allowed to settle and after being washed with normal salt solution collected on a filter of known weight. It is further washed with normal salt solution until free from coloring matter, then boiled in alcohol to dissolve out fat, cholesterin, and lecithin, dried at 110° to 120° C., and on cooling weighed over sulphuric acid.

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[1] Erben, Zeit. f. klin. Med., 1900, vol. xl, p. 266.

Fairly satisfactory results may also be obtained by simply making wet mounts (which see), ringing with vaselin and setting aside for several hours, when they are examined microscopically. In cases of pneumonia and acute articular rheumatism marked fibrin formation will be observed, starting from clumps of blood platelets.

The presence of albumoses and peptone bodies in the blood of leukemic (myeloid) patients has been repeatedly observed after the blood has stood for some time, or after the death of the patient (v. Jaksch.[1] Matthes,[2] Erben,[3] Schumm[4]). Their formation is due to the liberation of a proteolytic ferment, derived from the polynuclear neutrophiles. Schumm also found leucin and tyrosin. In normal human blood Schumm found no albumoses after death. In interstitial nephritis a fair amount could be demonstrated.

Albumoses have also been found in a case of abscess of the brain, associated with albumosuria. Freund[5] claims that they are met with in sarcoma, while they are absent in carcinoma (not confirmed).

Following the injection of nuclein and spermin albumosemia appears to occur quite constantly both during the stage of hypo- as well as hyperleukocytosis. After injections of pilocarpin albumosuria is observed only in association with hyperleukocytosis.

In order to test for albumoses, the coagulable albumins should first be removed, when a positive biuret reaction in the filtrate will indicate their presence (see also Salkowski's test).

Carbohydrates. Sugar.—Sugar, as indicated above, is a normal constituent of the blood, its quantity varying between 1 and 1.5 pro mille. Under pathological conditions this amount may be exceeded and notably so in diabetes, in which Hoppe-Seyler found as much as 9 pro mille in a given case.

In addition to sugar, a non-fermentable reducing substance has been encountered in the blood, which, according to Mayer, appears to be a compound glucuronate.[6] The presence of jecorin in the blood still remains to be proved.

Large quantities of a reducing substance, the greater portion of which consisted of sugar, have been met with by Trinkler in carcinoma; it was observed at the same time that carcinoma of internal organs was associated with far greater amounts of sugar than cancerous disease of the skin and the mucous membranes. It is also interesting to note in this connection that an increase in the degree of the cachexia was not accompanied by an increase in the percentage of sugar.

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[1] Zeit. f. physiol. Chem., vol. xvi, p. 243.
[2] Berlin. klin. Woch., 1894, Nos. 23 and 24.
[3] Zeit. f. Heilk., 1903, vol. xxiv.
[4] Hofmeister's Beit., vol. v, p. 442.
[5] Freund u. Obermayer, Zeit. f. physiol. Chem., vol. xv, p. 310.
[6] Ibid., vol. xxix, p. 59.

The results reached by Trinkler[1] apparently also bear out the correctness of the conclusions formed by Freund, who claimed that a differential diagnosis between carcinoma and sarcoma, in which latter condition no increase in the amount of sugar was noted, can always be effected upon the basis of an examination of the blood in this direction. Further examinations in this direction are lacking.

In the following table the percentages found in the different diseases investigated are given, from which it is apparent that, next to carcinoma, the largest quantities of sugar are met with in the infectious diseases and the lowest figures in diseases of the kidneys:

	Average. Per cent.	Minimum. Per cent.	Maximum. Per cent.
Carcinoma	0.1819	0.1023	0.3030
Typhoid fever	0.0950	0.0875	0.1022
Pneumonia	0.0943	0.0813	0.1092
Dysentery	0.0838	0.0796	0.0915
Heart disease	0.0737	0.0664	0.0897
Peritonitis	0.0701	0.0450	0.0917
Tuberculosis	0.0653	0.0450	0.0817
Syphilis	0.0553	0.0449	0.0748
Nephritis and uremia	0.0489	0.0321	0.0559

In order to demonstrate sugar in the blood, 15 to 30 grams, obtained by venesection or cupping glasses, are placed in an evaporating dish and treated with an equal weight of finely powdered sodium sulphate and a few drops of acetic acid. The mixture is brought to the boiling point and passed through a muslin filter as soon as the coagulum has become black and spongy, water having previously been added to the original volume. The filtrate is passed through Swedish paper. In the final filtrate the sugar is then estimated as described elsewhere (see Urine).

Cavazzani has drawn attention to another method of freeing the blood from proteids, which is said to be entirely satisfactory. To this end, 20 to 30 c.c. of blood are added to 200 c.c. of distilled water in a porcelain dish and treated with 5 or 6 drops of a solution consisting of 10 parts of acetic acid (sp. gr. 1.040) and 1 part of lactic acid. The mixture is boiled for eight to ten minutes, filtered, and the coagulum washed repeatedly with hot water and finally pressed out in a piece of muslin. The resulting filtrates, which are practically colorless, are then concentrated to a small volume, and any traces of albumin, which may still separate out, filtered off. If an excess of the acid solution has been added, it may happen that the mixture does not clear up on boiling. It is then only necessary to add a few crystals of sodium carbonate, when coagulation will occur at once. On the other hand, it may at times be necessary to add a few more drops of the acetic acid solution.

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[1] Centralbl. f. d. med. Wiss., 1890, p. 498. Freund u. Obermayer, loc. cit.

Williamson's Diabetic Blood Test.—This test is of much interest, and may possibly serve to differentiate the ordinary forms of diabetes from that in which the blood sugar is not increased. It is based upon the observation that a warm alkaline solution of methylene blue is decolorized by grape sugar. A positive result may at times be obtained when the sugar has temporarily disappeared from the urine.[1]

METHOD.—Twenty cbmm. of blood, obtained from the finger or the ear, are carefully measured off with the aid of the capillary pipette, which accompanies Gower's hemocytometer, and mixed in a test-tube of small caliber with 40 cbmm. of distilled water. To this mixture 1 c.c. of an aqueous solution of methylene blue (1 to 6000) and 40 cbmm. of a 6 per cent. aqueous solution of potassium hydrate are added. A control tube is similarly charged with non-diabetic blood. The two specimens are placed in boiling water and allowed to remain for three to four minutes, without shaking. At the end of this time it will be seen that the diabetic blood has decolorized the methylene-blue solution, which has turned a dirty yellowish green or yellow, while the non-diabetic specimen has retained its original color.

The quantity of blood used should not exceed the amount indicated, as a decolorization of the methylene blue also results with non-diabetic blood if large amounts, such as 60 cbmm., are employed.

The reaction is supposedly due to an increase of glucose in the blood, and was obtained in all of forty-three cases of diabetes which were examined. It is said to be obtainable for a considerable time after death. Adler[2] found the reaction in all of nine cases of diabetes, while in one hundred and twenty-one non-diabetic cases negative results were reached. Very curiously, it was absent in non-diabetic glycosurias. Adler believes the reaction to be referable to a diminished alkalinity of the blood.

Glycogen.—There appears to be no doubt that glycogen normally occurs in the blood of various animals. Huppert[3] succeeded in demonstrating its presence in all animals examined, the amount varying between 0.114 and 1.560 grams for 100 parts of blood (see Iodophilia).

Cellulose.—Cellulose has been found in the blood of tuberculous patients.

Urea.—Urea occurs normally in the blood in traces—0.016 to 0.020 per cent. Larger amounts are encountered whenever, as in nephritis, various diseases of the urinary organs, cholera Asiatica, cholera infantum, eclampsia, etc., its elimination is *impeded*, or whenever, as in fever, owing to increased albuminous decomposition, urea is *formed* in abnormally large quantities.

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[1] R. T. Williamson, Centralbl. f. inn. Med., vol. xviii, No. 33.
[2] Zeit. f. Heilk., 1900, vol. xxi, No. 11.
[3] Zeit. f. physiol. Chem., 1893, vol. xviii, p. 144.

It is interesting to note that a smaller amount of urea is found in fatal cases of eclampsia than in those ending in recovery, which has been explained by the assumption that in this condition the functional activity, not only of the kidneys, but also of the liver, is lost.

The methods which are available for the detection of urea in the blood are still too complicated for clinical purposes, and the value of the information derived so small as hardly to warrant the labor involved. Hoppe-Seyler's method should be employed whenever an examination in this direction is deemed advisable.[1]

Uremia.—Formerly, it was thought that the complex of symptoms generally spoken of as uremia was referable to the retention in the blood of urea or ammonium carbonate. This view has since been disproved, although it must be admitted that in uremia an increased amount of urea is frequently noted. Other views, according to which uremia is referable to an accumulation of potassium salts, of extractives, and especially of kreatinin, or of ptomaïns in the blood, must be regarded as being *sub judice*. There is no reason, however, to ascribe the uremic condition to the retention in the blood of one particular constituent of the urine, and it is not improbable that a retention of all may be responsible for the symptoms observed.

LITERATURE.—Feltz and Ritter, De l'uremie exper., Paris, 1881. Astaschewsky, St. Petersburg med. Woch., 1881, No. 27. Bouchard, Leçons sur l'autointoxication, Paris, 1887. Rovighi, Rivista clinica, 1886.

Ammonia.—Normal venous blood, according to the researches of Winterberg, contains about 1 mgrm. of ammonia for each 100 c.c. In febrile conditions variable results are obtained, but it appears certain that a definite relation between the height of the fever and the amount of ammonia does not exist. In chronic hepatic diseases, and notably in cirrhosis, it is not increased. Acute yellow atrophy also is not necessarily associated with an increase. Very significant is the observation that in uremia following extirpation of the kidneys no increase is observed. An ammoniemia in the sense of v. Jaksch can hence scarcely be said to exist.

LITERATURE.—Nencki, Pawlow, and Zaleski, Arch. f. exp. Path. u. Pharmakol., 1896, vol. xxxvii, p. 26. Winterberg, Wien. klin. Woch., 1897, p. 330.

Uric Acid and the Xanthin Bases. Uric Acid.—Formerly, the presence of appreciable amounts of uric acid in the blood was regarded as pathognomonic of gout. But we now know that a lithemic condition may occur also in other diseases. Traces of uric acid are indeed encountered under normal conditions.

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[1] See Hoppe-Seyler, Handbuch der physiologisch und pathologisch-chemischen Analyse.

A definite lithemia has been observed in a variety of disorders, such as pneumonia, acute and chronic nephritis, leukemia, conditions associated with an insufficient aëration of the blood, as in the various diseases of the heart, in pleurisy with exudation, emphysema when accompanied by cyanosis, the severer forms of anemia, etc. v. Jaksch claims to have found uric acid in the blood in 88.88 per cent. of his cases of nephritis. Fever in itself does not appear to lead to an increased production of uric acid, as negative results were obtained in nine cases of typhoid fever out of eleven, in five cases of acute articular rheumatism out of six, etc.

The assumption that acute attacks of gout are referable to increased alkalinity of the blood, and a consequent increase in the amount of circulating uric acid, has been disproved.

In order to estimate the amount of uric acid in the blood, the following method may be employed: 100 c.c. of blood, obtained by means of venesection or of cupping glasses, are at once diluted with three or four times their volume of water and heated on a water bath. As soon as coagulation sets in, a few drops of a 0.3 to 0.5 per cent. solution of acetic acid are added until a feebly acid reaction is obtained. After having been kept upon the boiling water bath for from fifteen to twenty minutes longer, until the albumin has separated out and settled in brownish flakes, the mixture is filtered while hot, and the precipitate washed repeatedly with hot water. Filtrate and washings, which usually present a slightly yellow or brownish color, are again brought to the boiling point after the addition of 0.3 to 0.5 per cent. of acetic acid, decanted, filtered, and after the addition of a small amount of disodic phosphate further treated according to Folin's method (see Urine).

LITERATURE.—Picard, Virchow's Archiv. vol. ii, p. 189. Garrod, Med.-Chir. Trans., 1854, p. 49. Salomon, Zeit. f. physiol. Chem., vol. ii, p. 65; and Charité Annalen, 1880, vol. v, p. 137. Klemperer, Deutsch. med. Woch., 1895, No. 40. Weintraud, ibid., V. B. p. 185.

Xanthin Bases.—Xanthin bases do not occur in normal blood or are present only in exceedingly small amounts. Under pathological conditions they may be encountered in recognizable quantities, so in leukemia, typhoid fever, lymphatic tuberculosis, emphysema, phthisis pulmonalis, pleurisy, and chronic nephritis.

To demonstrate the xanthin bases in the blood the albumins are first removed as just described (see Uric Acid) and the filtrate then examined according to Salkowski's method (see Urine).

LITERATURE.—A. Kossel, Zeit. f. physiol. Chem., 1882, vol. vii, p. 22. Scherer, Verhandl. d. physik. med. Ges. z. Würzburg, 1852, vol. ii, p. 325.

Fat and Fatty Acids.—Engelhardt has pointed out that the amount of fat which is contained in normal human blood may be subject to considerable variations, and gives 0.194 per cent. as the average. The lowest figure which he obtained was 0.101 and the highest 0.273 per cent. These figures differ very materially from those of older observers, who have found from 0.73 to 1.4 per cent., but it is quite likely that Engelhardt's method is responsible for these differences, and is probably more reliable (see below). Unfortunately only a few analyses of pathological material have been made with this method, and these have reference only to the blood of cachectic individuals. An increase in the amount of fat has here not been demonstrated, the results varying between 0.112 and 0.284 per cent., with 0.174 as an average. The cachexias in question were of tuberculous and carcinomatous origin. With the older methods an increase in the amount of fat, aside from that observed after the ingestion of large amounts of fatty food, has been met with in cases of obesity, chronic alcoholism, in phosphorus poisoning, in injuries affecting the long bones and the spinal cord, in various hepatic diseases, chronic nephritis, tuberculosis, malaria, cholera, during starvation, pregnancy, in nursing infants, etc. The greatest increase, however, is observed in certain cases of severe diabetes, in which amounts varying between 1.276 and 18.12 per cent. have been encountered, and in which the fat may be visible with the naked eye (see below). In such cases fat emboli may be found postmortem, plugging the vessels of various

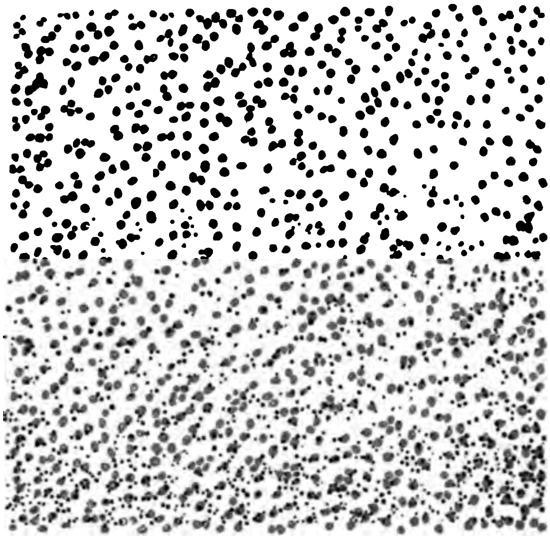

FIG. 12.—Pronounced lipemia. Specimen treated with osmic acid. Lower half shows extracellular fat globules, upper half having been cleared by oil of turpentine. (Gumprecht.)

organs, and notably the brain, the lungs, and the kidneys. This increase in the amount of fat constitutes the condition spoken of as *lipemia.*

The term lipacidemia has been applied to the occurrence of fatty acids in the blood. This has been noted in various febrile diseases, leukemia, and especially in grave cases of diabetes, where beta-oxybutyric acid may be found in large amounts, and is no doubt directly concerned in the production of coma.

To demonstrate the presence of fat in the blood, it is best to prepare cover-glass specimens, and to mount these in a drop of a 5 per cent. solution of osmic acid. The fat droplets are thus colored black, and appear about as large as the finest fat granules which are found in milk or butter. They may also be stained with Sudan III, or Biebrich scarlet, and are thus colored red. In every case the necessary instruments and glasses should be carefully cleansed with ether, so as to avoid the accidental introduction of fat.

As a quantitative estimation of the fat is not always possible, Landy recommends the following simple procedure to demonstrate the presence of an excess of fat: A small drop of blood is received upon a cover-glass, which is then adjusted over the depression of a cupped slide and ringed with vaselin. On standing, the serum separates out concentrically or excentrically from the small blood clot, and normally or in the presence of no excess of fat appears perfectly clear. If, however, much fat is present, it becomes cloudy after several minutes or hours, and then appears bluish-white, grayish-white, or even milky-white. To ascertain positively that the turbidity is due to fat, a microscopic examination of the hanging drop is made within a few hours following the preparation of the specimen, so as to exclude fibrin as the possible cause of such turbidity.

Quantitative Estimation.—The apparatus which is best used is a modification of that of Nerking, as suggested by Engelhardt.[1] As seen from Fig. 13, it consists of the ether flask *A*, which is placed on a permanent water bath, such as that of Münke. *a* represents the escape tube for the ether vapor; at *b* there is a closure by means of mercury, the upper escape tube *c* dipping into the mercury over the mouth of *b*. *B* is the cooler for the ether vapor; *C*, the water condenser. The cooled ether falls through the cooler into *d*. This ends below with a funnel-shaped mouth, close to the bottom of the extraction flask *E*, with five apertures, and has a small open side tube, *f*, which counteracts any negative pressure that may occur above the liquid in the extraction flask. The fluid to be extracted extends to within 1 to 2 cm. from the aperture of the off-flow tube *i*. When the ether layer extends to the level with *k* the tube *i* acts as a siphon and draws off the fatty ether into *A* again by way of the tube *l*, which is likewise provided with a mercury stop.

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[1] The apparatus may be procured from Arno Haak, Jena. Price, 12 marks.

The blood, about 10 c.c., is received in a graduate and weighed. It is washed into the extraction flask with about ten times its volume of 2 per cent. hydrochloric acid and boiled for three hours (with inverted condenser). On cooling, the material is extracted in the apparatus described for about forty-eight hours. At the expiration of this time the fatty ether in *A* is poured into a separating funnel together with the ethereal washings, which are used to remove all the material from the flask, the idea being to get rid of any water or bits of the bloody material that may by chance have been siphoned into *A*. The ether is then evaporated in an open glass dish. The residue is dissolved in absolute ether and filtered through a double folded filter (so as to absorb any traces of water remaining) into a beaker, when the ether is allowed to evaporate. The residue is placed in a drying oven at 40° C. for one hour, and after remaining in the vacuum over sulphuric acid for twelve hours it is weighed.

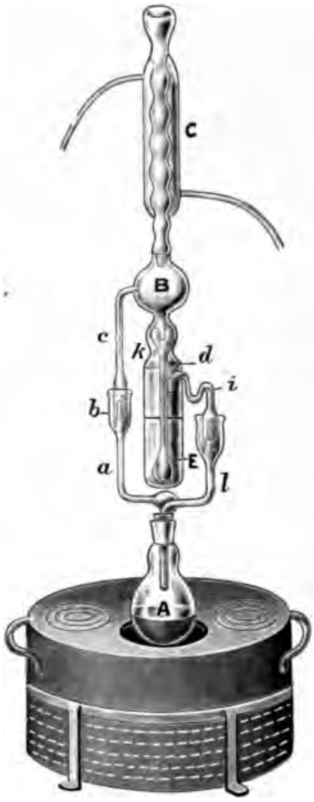

FIG. 13.—Fat-extraction apparatus.

With this method lecithins, cholesterins, and fatty acids are obtained conjointly with the fat, which Engelhardt does not regard as objectionable, as they are present only in traces and may be regarded as physiologically equivalent to neutral fat.

Estimation of Fatty Acids.—This is carried out along the same lines as described in the Urine (Lipaciduria), after removal of the coagulable albumins. At least 20 to 30 c.c. should be available.

Cholesterin.—Traces of cholesterin are normally met with in the blood. Larger amounts have been observed in diabetes (0.478 per cent.) in association with marked lipemia.[1]

Hale White[2] reports a case in which microscopic examination showed a granular precipitate, which did not stain with osmic acid. Chemical examination led to the conclusion that the substance was an ester of cholesterin with one or more of the higher fatty acids.

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[1] Virchow's Archiv, vol. clxxii, Heft 1 and 2.
[2] Lancet, October 10, 1903.

LITERATURE.—M. Bönninger, "On the Methods for the Estimation of Fat in the Blood, and the Amount of Fat in Human Blood," Zeit. f. klin. Med., vol. xlii, parts i and ii. T. B. Futcher, "Lipemia in Diabetes Mellitus," Jour. Am. Med. Assoc., 1899, p. 1006. S. Watjoff, "Ueber d. Fettgehalt d. Blutes b. Nierenkrankheiten," Deutsch. med. Woch., 1897, p. 559. v. Jaksch, "Lipacidæmie," Zeit. f. klin. Med., vol. xi. W. Ebstein, "Beitrag z. Lehre v. d. Lipemie u. d. Fettembolie," etc., Virchow's Archiv, 1899, vol. clv, p. 571. M. Engelhardt, Deutsch. Arch. f. klin. Med., 1901, vol lxx, p. 182. Zandy, ibid., vol. lxx, p. 301.

Lactic Acid.—There appears to be some doubt whether or not lactic acid normally occurs in the blood of man during life. In the blood of dogs, Gaglio, could always demonstrate the presence of the acid during the process of digestion, after feeding with meat. The amount varied between 0.3 and 0.5 pro mille. During starvation smaller amounts were found, but it never disappeared altogether. In one instance Gaglio obtained 0.17 pro mille after fasting for forty-eight hours. Similar results were obtained by Irisawa, who noted that the amount of lactic acid in the blood stood in direct relation to the degree of anemia which was produced.

In the human being Irisawa found lactic acid fairly constantly after death, the amount, determined as zinc lactate, varying between 0.233 and 6.575 pro mille. These extensive variations he was unable to explain by the character of the disease causing the fatal termination, and it is possible that the cause lies in the fact that in some cases the blood was obtained shortly after death, while in others many hours had elapsed, as Irisawa himself suggests.

The following method may be employed: 100 to 300 c.c. of blood are extracted with three times its volume of alcohol, filtered, and the filtrate evaporated to a syrupy consistence. This is then made strongly alkaline with barium hydrate and shaken with large quantities of ether, in order to remove the fats which are present. The residue is acidified with phosphoric acid and again shaken with ether for twenty minutes at a time, until the process has been repeated five or six times, the lactic acid passing over into the ether. The ether is distilled off from the extract, the residue taken up with water, and the solution carefully evaporated in order to drive off any ether still remaining, as well as the fatty acids. Carbonate of zinc is now added and the solution heated to 100° C. and filtered. The filtrate is evaporated on a water bath until crystallization begins, when it is allowed to cool and treated with a few drops of absolute alcohol, in order to effect a complete separation of the lactate of zinc. The solution is allowed to stand exposed to the air until a constant weight is obtained.

LITERATURE.—G. Gaglio, "Die Milchsäure d. Blutes," Du Bois Archiv., 1886, p. 400. T. Irisawa, "Ueber d. Milchsäure im Blut und Harn," Zeit. f. physiol. Chem., 1892, vol. xvii, p. 349.

Homogentisinic Acid.—Homogentisinic acid has been demonstrated in the blood serum of an alkaptonuric, by Abderhalden and Falta.[1]

Biliary Constituents and Urobilin.—Bile pigment does not occur in the blood under normal conditions, but may be demonstrated whenever it is present in the urine (obstructive jaundice, hepatic cirrhosis, acute yellow atrophy, phosphorus poisoning, etc.). It appears, moreover, that bilirubin is present in the blood in nearly every case where urobilin is found in the urine. In pernicious anemia bilirubinemia is thus quite constantly associated with urobilinuria. At the same time urobilin can usually be demonstrated in the blood.

In chlorosis bile pigment does not occur in the blood.

The demonstration of bilirubinemia constitutes the most delicate test for the entrance of bile into the blood and lymph; it is a much more delicate indication than the occurrence of bilirubinuria.

Bilirubin can be demonstrated in the blood most readily in the following manner: A short piece of glass tubing is drawn out so as to form a tapering lower end, which is then sealed. By means of a pipette 10 to 15 drops of blood, obtained by free puncture of the finger or ear, are transferred to the first tube and the serum separated from the corpuscles by centrifugation. The coagulum which forms is separated from the walls and packed down into the lower portion of the tube. The supernatant fluid is normally clear or but faintly turbid, and of a straw color: in the presence of bilirubin it is colored a bright yellow, which on exposure to the air gradually turns to a greenish tint.

For more exact information the method of Syllaba may be used: 10 to 15 c.c. of blood are placed in a cool place for sedimentation. The serum which separates out is removed with a pipette and 5 c.c. diluted with double the amount of water and coagulated by boiling after the addition of a pinch of sodium sulphate and acidifying with dilute acetic acid. Any bilirubin that may be present is carried down in the coagulating albumin while urobilin remains in solution. The fluid is then filtered and the filtrate tested by boiling to make sure that the coagulation is complete.

If no urobilin is present the filtrate is clear, colorless and spectroscopically free from absorption; if, however, urobilin is present in the serum, as is usually the case in pernicious anemia, then the filtrate presents a reddish color and shows a narrow band of absorption between *b* and *F*. The collected precipitate in the absence of bilirubin (in normal serum and the serum of chlorosis) is white, but in the presence of bilirubin (in the serum of pernicious anemia) of a slight yellowish color. The precipitate is washed with hot water, boiled with acidulated alcohol (sulphuric acid) and the mixture filtered. In the presence of bilirubin the alcohol is colored a fine green and the coagulum presents the same color; in the absence of bilirubin the alcohol remains colorless.

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[1] Zeit. f. phys. Chem., vol xxxix, p. 143.

In order to *test for biliary acids*, the blood is first treated with alcohol, in order to remove the proteids. The biliary acids which are present in the filtrate are next transformed into their lead salts by means of lead acetate and ammonia and thus precipitated. After washing with water the precipitate is boiled with alcohol and filtered. The lead salts are decomposed by means of sodium carbonate, the solution is again filtered, the filtrate evaporated to dryness, and the residue extracted with absolute alcohol. The alcohol is distilled off, when the biliary salts of sodium will crystallize out or remain behind as an amorphous mass, which may be tested directly according to Pettenkoffer's method. To this end, some of the residue is dissolved in water and treated with two-thirds of its volume of concentrated sulphuric acid, care being taken that the temperature does not rise beyond 60° C. To this mixture a few drops of a 20 per cent. solution of cane sugar are added, when in the presence of biliary acids a beautiful violet color is obtained, which is referable to the action of furfurol, formed from the cane sugar and the acid, upon the biliary acids.

Acetone.——Acetone has been found in the blood in considerable amounts under various pathological conditions, and especially in diabetes and fevers.

In order to demonstrate its presence, *Dennigè's test* may be employed: 3 c.c. of blood are treated with about 30 c.c. of Dennigè's reagent and allowed to stand until the dark-brown precipitate has settled to the bottom. The supernatant fluid is filtered off and treated with a little more of the reagent, so as to ensure *complete* precipitation. It is then acidified with sulphuric acid and heated as described. The formation of a white precipitate, which is soluble in an excess of hydrochloric acid, is referable to acetone or diacetic acid. (See Urine.)

LITERATURE.—v. Jaksch, Acetonurie u. Diaceturie, Berlin, 1885. Reale, Schmidt's Jahrbüch., 1892, p. 106 (Extract).

Cholin.—Cholin has been demonstrated by Moth and Halliburton in the blood in diseases of the nervous system which are associated with a destruction of nerve tissue; notably in *general paresis, tabes,* combined sclerosis, disseminated sclerosis, alcoholic polyneuritis, beriberi, and following the division of both sciatic nerves in cats,

METHOD.—Five c.c. of blood are treated with from six to eight times that amount of absolute alcohol and filtered. The filtrate is dried at 40° C., and the *dry* residue extracted three times with *absolute* alcohol, filtered, and the solution evaporated. The alcoholic solution of the residue is precipitated with a 10 per cent. alcoholic solution of platinum chloride and the precipitate decanted from the absolute alcohol. The precipitate is finally dissolved in 15 per cent. alcohol, the solution filtered and evaporated in a watch crystal at 40° C. With a low power the octahedral crystals of cholin-platinochloride can then be seen.

Normal human blood (in the amount mentioned) rarely gives rise to such crystals, so that the result is practically negative. *Sine qua non* for the success of the method is that the alcohol is absolute; 99 per cent. will not suffice. [See also Donath's method (Cerebrospinal fluid)].

MICROSCOPIC EXAMINATION OF THE BLOOD.

The Red Corpuscles. Variations in Size and Form.—The normal red blood corpuscles are greenish-yellow, circular bodies, which in postembryonic life are non-nucleated. While it has been generally accepted that the red cells are biconcave several writers have recently insisted that they are bell-shaped (Weidenreich, F. T. Lewis). They are possibly composed of fluid contents within a membrane of some fatty substance. Their diameter varies between 6 and 9 μ, with an average of 7.5 μ. The presence of larger or smaller cells is abnormal. Smaller cells are termed *microcytes* and measure from 3.5 to 6 μ; larger cells are known as *macrocytes* or *megalocytes*, and usually have a diameter of from 9.5 to 12 μ; still larger specimens are spoken of as giant corpuscles (Hayem);[1] they may attain a diameter of 16 μ. The terms *microcytosis* or *microcythemia* and *macrocytosis* or *macrocythemia* are used to designate a predominance of the corresponding variety.

As regards the origin of the macrocytes, there is evidence to show that they may result from the common normocytes in the circulating blood through imbibition of water, so that their occurrence from this point of view could be regarded as a degenerative phenomenon. But, on the other hand, the presence of macrocytes may be interpreted as evidence of a regenerative process, bearing in mind that in the bone-marrow the size of the erythroblasts is larger than that of the common normocytes; the macrocytes would thus represent young normocytes which have prematurely found their way into the circulation. The microcytes probably result from the normocytes in the circulating blood through loss of water; whether their presence may at any time be regarded as the expression of a regenerative process seems questionable. Not infrequently microcytes are formed artificially during the preparation of the specimen.

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[1] Le Sang, Paris, 1891.

Microcytosis is, on the whole, of comparatively little clinical interest, and may be observed in any severe anemia. Macrocytosis is more important. To a certain extent it is seen in severe forms of anemia of whatever origin, but it is noteworthy that the presence of macrocytes in large numbers is essentially observed in pernicious anemia. During the active period of the disease the macrocytes may here represent 70 per cent. of all red cells (Lazarus). The condition, however, is not constant.

Going hand in hand with pathological variations in the size of the red corpuscles—*anisocytosis*—there are variations in form which may affect not only the microcytes and macrocytes, but also the corpuscles of normal size. Cells may thus be seen which resemble a flask, a kidney, a biscuit, a boat, a balloon, a dumb-bell, or an anvil, while others are altogether irregular in appearance.

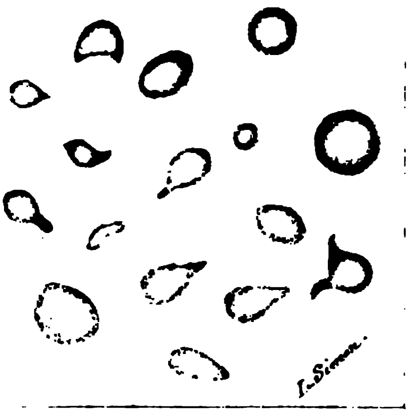
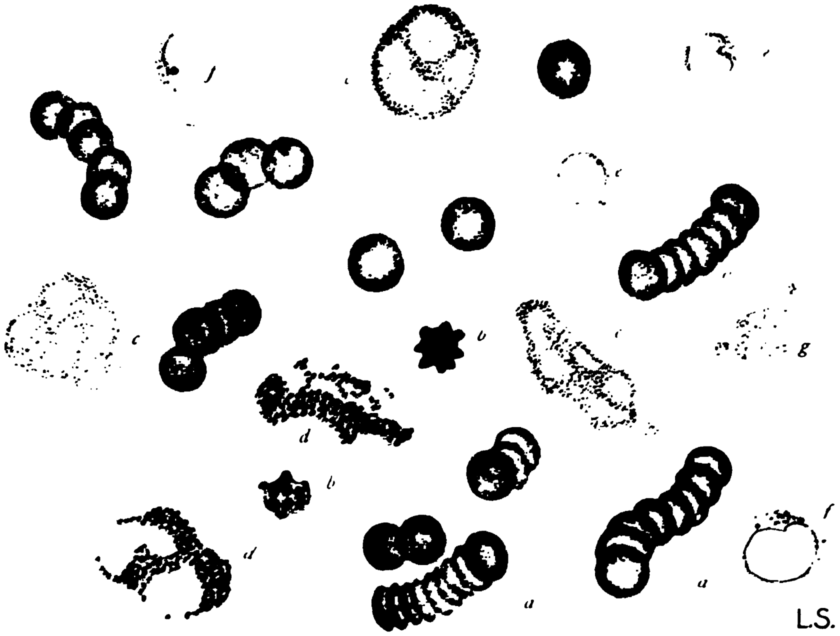


FIG. 14.—Poikilocytosis.

Especially interesting is the fact that such abnormally formed cells, which are generally spoken of as *poikilocytes* (Fig. 14), may manifest a certain degree of motility, so that they have at times been mistaken for microparasites. This is seen especially in marked cases of pernicious anemia, and is most noticeable in the smaller forms. In pernicious anemia *poikilocytosis* is most pronounced, and at one time it was thought that the condition was characteristic of the disease. It has been shown, however, that it occurs in other anemias as well, though its occurrence is probably always evidence of a specially severe form. In chlorosis it is usually only seen in the most severe cases, and particularly in those manifesting a tendency to thrombosis and embolism.

In this connection a special deviation from the normal form of the red corpuscles also requires consideration, viz., the prevalence of

PLATE II.



The Elements of Normal Blood.

a, red cells in rouleaux; *b*, crenated red cells; *c*, finely granular (neutrophilic) leukocytes; *d*, coarsely granular (eosinophilic) leukocytes; *e*, small, and *f*, large mononuclear leukocytes; *g*, plaques.

oval cells. These are notably observed in pernicious anemia and seem to be of distinct diagnostic importance. They are found not only during the active periods of the disease, but frequently also in the interval between exacerbations.

Poikilocytosis is a degenerative phenomenon, and it is essential not to confound true poikilocytes with certain abnormal forms, which may be seen in any normal preparation and which are the result of mechanical injury, mutual compression, etc., and can readily be distinguished with practice.

In wet preparations red cells will be seen near the margin of the drop where evaporation is actively going on, which present little knobs or spicules on their surface and along the periphery. Such cells are spoken of as crenated cells. The phenomenon in itself is normal, but it is noteworthy that *crenation* may at times be observed in the centre of a carefully prepared specimen after a few seconds already, while as a rule from fifteen to thirty minutes elapse before the process begins to attack cells in this location. The significance of this early crenation is not known. This is also true of delayed *money-roll formation,* which is observed in various hepatic diseases, in pneumonia, nephritis, etc., whereas normally the red corpuscles tend to agglutinate in this form immediately unless special pains have been taken to secure the separation of the individual cells. (See Plate II.)

Variations in the Color of the Red Corpuscles.—The degree of coloring of the red corpuscles depends upon the amount of hemoglobin. The centres of the cells in well-mounted specimens are always paler than the periphery, and any deficiency in the amount of coloring matter is here at once apparent. With a moderate grade of anemia the cell as a whole looks paler, and the pale central area is increased in size. With a further increase in the loss of coloring matter the central area is absolutely colorless and encroaches upon the peripheral colored zone more and more until finally the so-called *pessary forms* result, in which only a narrow rim of hemoglobin remains. These changes can be made out in wet preparations, but are especially well seen in stained specimens. The central pale area is, however, visible only in well-preserved cells and not in flattened out cells, which are stained uniformly throughout and which may also be seen in any specimen.

The color of the normal red cells in wet specimens is a pale greenish-yellow. In malaria curiously discolored corpuscles are seen, which present a bronzed appearance; their presence should always excite suspicion. The meaning of the discoloration is not known, but in all probability it is evidence of a degenerative process.

The Color Index.—The term color index is used to designate the relative amount of hemoglobin which is contained in each corpuscle. It is determined by dividing the percentage of blood coloring matter by the percentage of red cells as compared with the recognized normal, viz., 5,000,000.

Example.—The percentage of hemoglobin is 50, the red count per cbmm. is 2,000,000, viz., 40 per cent. of the recognized normal, 5,000,000. The color index is then 50 divided by 40—*i. e.*, 1.25.

Under normal conditions the color index is about 1, but may vary from 0.95 to 1.17; it is slightly higher in men than in women. In the secondary anemias, in which the decrease in the amount of hemoglobin is proportionate to the diminution of the red corpuscles, the color index is approximately normal. But in the majority of cases the diminution of the hemoglobin somewhat exceeds that of the red cells, so that lower values are commonly met with. In pernicious anemia, on the other hand, where the corpuscular decrease usually exceeds the diminution of the hemoglobin, a high color index is the rule. There may be periods in the course of the disease, however, in which a normal index and even subnormal values are found. In the chronic cases lower figures are more commonly obtained than in the acute cases. In the series of 22 cases collected by Strauss and Rohnstein the value of the color index varied between 0.5 and 1.95. In 8 cases of the series variations from 1.13 to 1.95 were observed, and in 6 lower values than 1 were noted, viz., 0.5 to 0.9. Cases in which the color index falls as low as 0.5 are rare in pernicious anemia. In the one instance in the series in which this was found, the hemoglobin was only 10 per cent., while the red cells numbered 1,048,000; there was a high grade of poikilocytosis and all transitions between the smallest microcytes and the largest types of macrocytes.

In well-established hookworm anemia in contradistinction to the cryptogenetic type of pernicious anemia the color index is low (Ashford).

In the secondary anemia of carcinomatosis the color index rarely exceeds 1. In Strauss and Rohnstein's series[1] of 35 cases the highest value was 1.1 (in one case only); in the rest it varied between 0.53 and 0.96.

In chlorosis, in which the degree of oligochromemia exceeds the corpuscular loss the color index is markedly lowered; in especially severe cases it may fall to 0.3 and even lower. But it is not admissible to make the diagnosis of chlorosis on this basis only, as it is fairly common to meet with a markedly lowered color index in some secondary anemias also, and especially in the form which is referable to carcinoma, as has just been mentioned. In splenic anemia likewise the degree of oligochromemia may far exceed the degree of oligocythemia.

Variations in Number.—The number of red corpuscles in the blood of healthy adults is fairly constant. In man 5,000,000 may be considered a fair average, and in women 4,500,000. Higher values are not uncommon, but rarely exceed 6,000,000 in perfectly normal individuals.

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[1] Die Blutzusammensetzung b. d. verschiedenen Anemien. Berlin, 1891.

The largest number is found on the first day after birth, average 6,985,428. It diminishes until the third day. Following a temporary rise it drops farther and becomes fairly constant between the sixth and the tenth day.[1]

In 20 healthy infants Karnizki[2] obtained the following values:

Age.	
2–4 months	5,239,725
4–8 "	5,703,000 to 5,843,000
8–12 "	5,531,000 to 5,590,521

Then the number increases, especially after the sixth year, and remains on an average higher during childhood than in babyhood.

A somewhat higher average is found among people living at a considerable elevation above the sea level, and it is interesting to note that an increase in the number occurs whenever a change in the habitation is made from a lower to a higher level. This increase is frequently quite marked, as is apparent from the following table, which is taken from Ehrlich:[3]

Altitude	Increase of
561 meters	800,000
700 "	1,000,000
1800 "	2,000,000
4392 "	3,000,000

A corresponding diminution occurs when a change is made from a higher to a lower level.

In this connection Gaule's[4] observations are of interest. On the occasion of a balloon ascension to a height of from 4200 to 4700 meters he counted 7,040,000, 8,800,000, and 7,480,000, respectively, in the three participants of the journey. The hemoglobin was at the same time diminished, and he accordingly concluded that the increase during the ascent was due to an increased production of red cells; the probable nature of this conclusion was strengthened by the fact that numerous normoblasts were found in the blood, many undergoing division. Jolly and Bensaude[5] and others on similar expeditions were unable, however, to demonstrate the presence of nucleated red cells or to note the occurrence of an increased number of red cells. According to Weinzirl,[6] the increased counts due to high altitude are temporary and in part at least referable to cold. He showed that in rabbits a certain increase in the number of red cells occurs when they are removed from warm to cold quarters, and that their subsequent removal to a higher altitude does not lead to a further increase.

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[1] Scipiades, Arch. f. Gyn., vol. ixx, p. 630.

[2] Arch. f. Kinderheilk., 1903, vol. xxxvi.

[3] "Die Anämie," Nothnagel's specielle Path. u. Therap., vol. viii, part. i.

[4] Compt.-rend., vol. cxxxiii, p. 903.

[5] Compt.-rend. Soc biol., vol. liii, p. 1084. Saint Martin, Soc de biol., July 23, 1904.

[6] Amer. Jour. Med. Sci., 1903, vol. cxxvi, p 299.

Clinically we distinguish between *relative polycythemia* in which the condition is due to a diminution in the quantity of plasma, and *true polycythemia* in which there is an actual increase in the number of the red corpuscles. Relative polycythemia is much the more common. In some instances it is due to loss of liquid by sweating, diarrhea, or increased diuresis. In another group of cases there is loss of liquid by secretion or transudation, as in obstruction of the pylorus with dilatation of the stomach, and in the constant loss of liquid from the blood in recurring ascites. In some of these cases the polycythemia is of high grade, and may persist for years. In advanced cases of nephritis, phthisis, malignant disease, etc., there is also a certain grade of relative polycythemia, due to loss of water from the body at large. The polycythemia which is noted in poisoning by phosphorus and carbon monoxide (in one case of coal-gas poisoning a count of 11,200,000 is reported), various coal-tar products, during and immediately after the administration of ether, following cold baths and severe muscular exercise, also belongs to this order and is no doubt referable to vasomotor disturbances. Of similar origin probably is the polycythemia which is noted in disease of the adrenal glands, where counts of from 6,000,000 to 7,000,000 have been repeatedly noted; and the same is probably true of diabetes, in which polcythemia may be observed both while fasting and while much fluid is being ingested.

True polycythemia is met with in diseases in which there is difficulty in proper aëration of the blood, as in heart disease,[1] and in a peculiar type of chronic cyanosis which has been described by Osler[2] as a new clinical entity, the so-called autotoxic enterogenous cyanosis. In acquired heart disease with continued inadequacy of the circulation of slight degree a moderate grade of polycythemia is very common; in the congenital form the figures often reach 8,000,000 to 9,000,000. The highest values are seen in Osler's disease, however. In the first nine cases which have been reported the highest count was 12,000,000; in eight it was above 9,000,000, and in the ninth it was 8,250,000. The usual range of hemoglobin at the same time was from 120 to 150; the specific gravity varied between 1.067 and 1.083, and the leukocyte count between 4000 and 20,000; as a rule it was below 10,000. Vaquez[3] notes that whereas in congenital heart disease and the coincident polyglobulism the diameter of the red cells is increased from 7.5 to 8.5, this is not observed in the idiopathic form of cyanosis.

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[1] Stengel, Proc. Path. Soc. Phila., 1899. Oertel, Deutsch. Arch., vol. i, p. 293.

[2] "Chronic Cyanosis with Polycythemia," Amer. Jour. Med. Sci., 1903, vol cxxvi, p. 187.

[3] Soc. biol., 7 Mai, 1892

While there can thus be no doubt that a true polycythemia does occur, it has been conclusively demonstrated that such a condition does not exist in what is generally termed *plethora*, and that the various symptoms of plethora formerly attributed to a general increase in the amount of blood are referable to vasomotor disturbances.

Oligocythemia, viz., a diminished number of red cells, is much more common than polycythemia. It may be temporary or permanent, and is seen in all forms of anemia of whatever origin. It is most marked in pernicious anemia. The exact figure will here, of course, depend upon the stage of the disease and the individual case. A decrease to one-half of the normal number may be seen in comparatively mild cases; a million red cells is a common count. The number may fall to 500,000 and even lower. In one case reported by Quincke[1] a count of 143,000 was observed, and it is interesting to note that seventy-four days later the same patient had 1,234,000 per cbmm. Osler[2] reports a case in which shortly before death the red cells fell below 100,000. This is the lowest count that has been recorded. In the stage of amelioration they may rise to 4,000,000 and even higher. In the series collected by Strauss and Rohnstein[3] 1,240,000 was the average at the time when the patient first came under observation, and in Cabot's series of one hundred and ten cases the average number is almost identical—1,200,000.

In chlorosis, contrary to what is found in pernicious anemia, the red cells are usually not much diminished. In Cabot's[4] series of seventy-seven cases the average count was 4,050,000. At times, however, cases are met with in which the diminution of the red cells almost keeps step with the diminution in the amount of hemoglobin. Von Limbeck cites three cases with 1,750,000, 1,850,000, and 1,930,000, respectively; and Hayem mentions an instance in which only 937,360 cells were counted. Such cases are exceptional.

As in chlorosis so also in splenic anemia, the corpuscular anemia is of very moderate grade, even though the diminution in the amount of hemoglobin may be considerable. Of the forty-one cases collected by Osler, the average was 3,425,000; the lowest count was 2,187,000 and the highest 5,200,000.

A similar condition is found in Kala-azar, where the number of red cells is commonly reduced to 2,000,000 to 3,000,000.

In leukemia the red cells are usually not diminished to a very great extent; and the oligocythemia is generally more marked in the lymphatic than in the myelogenous variety; the average figures in Cabot's series are 2,730,000 and 3,120,000, respectively. Counts of 1,000,000 or thereabout may, however, be met with.

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[1] Centralbl. f. d. med. Wiss., 1877, No. 47; and Deutsch. Arch., 1877, vol xx.

[2] Johns Hopkins Hosp. Bull., 1902, vol. xiii, p. 251.

[3] Loc. cit.

[4] Clinical Examination of the Blood, Wm. Wood & Co.

In pseudoleukemia the red cells may be only moderately diminished, viz., between 3,000,000 and 4,000,000, but in some cases the corpuscular destruction is quite active, and in the last stages of the disease values may be found which are not much above 1,000,000 or 1,500,000.

The count which is obtained in post-hemorrhagic cases will depend very largely upon the amount of blood lost and the time at which the examination is made. The lowest counts, according to Lyon,[1] Hühnerfauth,[2] and Siegel-Maydl,[3] are found between the second and the eleventh day. In Rieder's[4] case the figures varied between 1,300,000 and 3,335,000; in those of Strauss and Rohnstein,[5] between 1,119,000 and 4,420,000. A sudden reduction in the number to 1,000,000 or less is usually followed by a fatal result.

In the anemias of infancy and early childhood the oligocythemia is often very pronounced. In the infantile pseudoleukemia of v. Jaksch especially low values may be found associated with an increase of the leukocytes of such extent that the ratio between the two may be suggestive of true leukemia; there is, however, no myelemia, but an increase of the normal types. In infantile leukemia of the lymphatic variety McCrae found 2,350,000 as the highest count.

An extreme and rapidly progressive anemia is frequently noted in acute streptococcus infections. Grawitz[6] states that according to Rocher's investigations it is probable that the diminution of the red cells in septicemia is greater than in any other infectious disease and appears in a shorter time. Cases may indeed be encountered in which the question of pernicious anemia may enter into the diagnosis, as occurred in two cases of gonorrheal endocarditis which were observed by Osler.

An extreme grade of corpuscular destruction is also noted in malaria. In acute cases the loss of red cells during the first twenty-four hours may reach 1,000,000, and in two days even 2,000,000. In neglected chronic cases the count usually varies between 3,000,000 and 4,000,000; the oligocythemia may, however, be far more extensive, and Ewing[7] cites a case observed by Kelsch, with only 583.000 red cells per cbmm.

The anemia observed after typhoid fever is as a rule not very severe, but exceptional cases occur in which the loss of red corpuscles is considerable. Osler cites an instance in which the number fell to 1,300,000.

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[1] Virchow's Archiv, 1881, vol. xciv.
[2] Ibid., vol. lxxvi.
[3] Wien. med. Jahrbuch., 1884.
[4] Beit. z. Kenntniss d. Leukocytose, Leipzig, 1892.
[5] Loc. cit.
[6] Klin. pathol. d. Blutes, Enslin, Berlin, 1902.
[7] On the Blood, Lea Bros. and Co., 1901.

The post-rheumatic anemia is usually not so pronounced.

In acute endocarditis Stengel[1] has noted a rapid decrease of the red corpuscles, often to 50 and even 40 per cent.

In pulmonary tuberculosis the number of the red corpuscles runs a course parallel to that of the hemoglobin. Oligocythemia is really only seen during the third stage (2,000,000 to 2,500,000); while during the second stage, owing to an actual concentration of the blood (Grawitz), normal figures are the rule. In the first stage a diminution of their number (3,800,000) is only seen in patients who have repeatedly suffered from tuberculous affections (scrofula) since childhood, and in whom the onset of the pulmonary disease has been gradual, while, on the other hand, normal values are found in individuals who appear to be in perfect physical health, who are well nourished, with well-shaped chests, and without hereditary predisposition (Appelbaum).[2]

In acute gastritis, and usually in chronic gastritis also, the number of the red corpuscles is not diminished, while in carcinoma a marked oligocythemia occurs at some time in the course of the disease. In the earlier stages, however, this is often but little marked, and at times an apparent increase of the red cells is noted (relative polycythemia). Later a diminution is probably always found. In the severer forms of chronic gastritis a diminution is also fairly constant, but rarely so marked as in carcinoma, if we except those cases of gastric anadeny which present the clinical picture of a pernicious anemia. In the differential diagnosis between carcinoma of the stomach and pernicious anemia a count below 1,000,000 points to the latter disease. In ulcer of the stomach anemia of the chlorotic type is very common. In Cabot's series of 51 cases, 42 (80 per cent.) had a hemoglobin value of less than 50 per cent., and in the Hopkins series, reported by Futcher, the average value was 58 per cent., with a red count of 4,071,000. When hemorrhages have recently occurred the blood count may of course be very low.

In the majority of cases of rickets there is no material diminution in the number of the red cells, while the hemoglobin may be much reduced, but in the severer forms with visceral complications there may be oligocythemia of extreme grade. v. Jaksch cites a case in which the red count fell from 1,600,000 to 750,000 within three months, and Luzet noted a drop to 500,000 within three weeks (Ewing).

In congenital syphilis the oligocythemia is usually marked, excepting in very mild cases, and in the severer infections the blood picture may simulate that of pernicious anemia.

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[1] Loc. cit., p. 59.

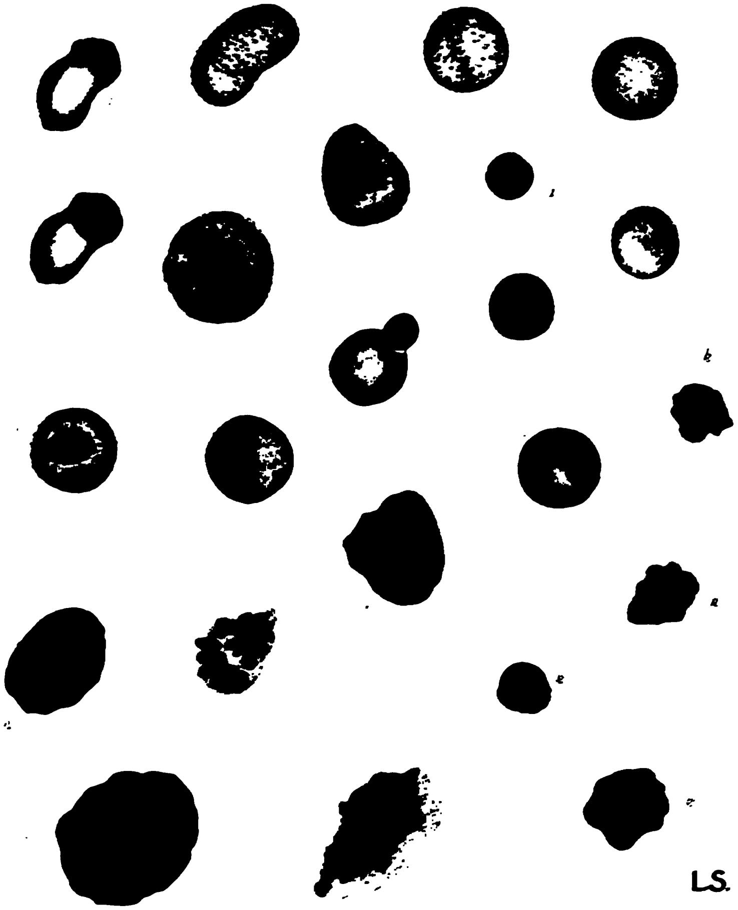
[2] Berl. klin. med. Woch., 1901, vol. xxxix, p. 7.

Behavior toward Aniline Dyes. Polychromatophilia (Polychromasia).—The normal *living* red cell possesses no affinity for dyes; it is achromatophilic. The normal *fixed* cell of the circulating blood, on the other hand, has a marked affinity for acid dyes, such as eosin, orange-G, acid fuchsin, etc.; it is accordingly said to be oxyphilic, and as it takes up only one color from a mixture of different dyes it is termed monochromatophilic. Under various pathological conditions which are associated with a marked grade of anemia cells are met with which are polychromatophilic. Such cells manifest an affinity not only for acid dyes, but simultaneously also for basic dyes, so that with a mixture of hematoxylin and eosin, or eosin and methylene blue, the red cells are not stained in the usual tint of the hemoglobin, but present a mixed color in which the tint of the basic dye is more or less apparent (Plate III).

As regards the significance of the polychromasia, Ehrlich maintained that the condition was evidence of a degenerative process—of a coagulation necrosis of the discoplasm as a consequence of which this takes up albumins from the blood plasma, while it loses the power of holding its hemoglobin. The oxyphilia hence diminishes, while owing to the absorption of albumins a more or less well-marked basophilia develops. As a matter of fact polychromatophilia is often seen in cells which are manifestly degenerating, and in myelogenous leukemia especially one frequently meets with nucleated red corpuscles which are markedly polychromatic and in which the protoplasm is evidently undergoing destruction, often appearing merely as a little hood attached to one side of the nucleus (see Plate III). Ehrlich accordingly speaks of an *anemic or polychromatophilic degeneration* of the blood. But, on the other hand, there is evidence to show that polychromasia may be the expression of a regenerative process, and we find as a matter of fact that the erythroblasts of the normal bone-marrow are for the most part polychromatophilic, and the more markedly so the younger they are. Megaloblasts are probably always polychromatophilic (Plate III). Welker has shown that basophilic red cells are normally found in pigeons, mice, guinea-pigs, cats, and dogs, while they are absent in the horse and the ox. I have also found them in the blood of birds, reptiles, amphibia, and fishes. In those animals, moreover, in which the red cells of the circulating blood are normally nucleated a certain grade of polychromasia, according to my experience, appears to be the rule in all the younger cells; the pure hemoglobin tint is only found in the mature forms.

Of late, Ehrlich has admitted the existence of a physiological polychromasia, but he still maintains that it may also occur as the expression of a degenerative process.

PLATE III.



[illegible] a group of red cells showing granular degeneration; [illegible] red cells showing Cabot's ring bodies; [illegible] normoblasts with nuclei undergoing karyolysis; the bodies of the cells show granular degeneration; [illegible] normoblasts with pyknotic nucleus; [illegible] red cell, suggesting loss of nucleus by extrusion; [illegible] red cell undergoing mitosis; h, megaloblasts with polychromasia of protoplasm; [illegible] gigantoblast; [illegible] megaloblasts showing wheel-like arrangement of the chromatin; [illegible] a group of platelets.

LITERATURE.—Ehrlich, Charité Annalen, vol. x, p. 136. Engel, Deutsch. med. Woch., 1899, p. 209. Gabritschewsky, Arch. f. exp. Path., vol. xxviii, p. 83; Zeit. f. klin. Med., vol. xxvii, p. 492. Askanazy, ibid., vol. xxi, p. 415. Maragliano and Castellino, ibid., vol. xxi, p. 415.

Diabetic Chromatophilia.—Bremer has pointed out that a distinct difference exists in the affinity of diabetic blood for certain aniline dyes, as compared with non-diabetic blood. For, whereas non-diabetic blood is readily stained with Congo-red, methyl blue, eosin, etc., diabetic blood is distinctly refractory, while such dyes as Biebrich scarlet, which readily stain the diabetic blood, do not color non-diabetic blood.

Regarding the nature of the substance in diabetic blood which is responsible for this peculiar behavior, little is known, but it appears certain that the reaction is not dependent upon the presence of glucose nor upon the degree of alkalinity of the blood, as suggested by Lépine and Lyonnet. Bremer's claim that the reaction is pathognomonic of diabetes and glucosuria and may even yield positive results in the pre-diabetic stage of the disease, and when the sugar has temporarily disappeared from the urine, has been confirmed in all essential points, both in this country and abroad. A few interesting exceptions, however, have been noted. In animals, for example, in which glucosuria has been artificially produced by means of phlorhizin, the reaction does not occur, whereas in phloroglucin-diabetes positive results are obtained. In Bremer's entire series of diabetic cases a negative result was obtained but once, and in this instance he believes that the diabetes was of the renal type, and analogous to the phlorhizin-diabetes of animals. He suggests that it may thus be possible to differentiate this form from the hematogenic variety, using the latter term in its widest sense. Lépine and Lyonnet report a positive result in one case of leukemia, but Bremer believes this to have been due to faulty technique. Hartwig finds that Bremer's reaction is constant in diabetes, but that it may also occur at times in other conditions.

The description of Bremer's diabetic blood test is omitted at this place, as it has not proved practical for routine work.

For a consideration of the technique see the Literature below.

LITERATURE.—L. Bremer, "An Improved Method of Diagnosticating Diabetes from a Drop of Blood," N.Y. Med. Jour., 1896; Centralbl. f. inn. Med., 1897, p. 521. Le Goff, React. chrom. du sang diabet., Paris, 1897. Lépine and Lyonnet, Lyon méd., vol. lxxxii, p. 187. Hartwig, Deutsch. Arch. f. klin. Med., vol. lxii, p. 287.

Granular Degeneration of the Red Cells.—Under pathological conditions red cells may be met with which contain basophilic granules. These are readily stained with methylene blue, methylene azure, thionin, etc. Methyl green, however, which is a specific unclear dye, does not stain the granules. Their size, form, and number are variable. While the majority are round, others are rod-shaped or biscuit-shaped. The largest granules are found in pernicious anemia and in cases of lead poisoning with intestinal manifestations. They are then quite readily seen and attract attention at once (Plate III). In most other diseases in which they occur they are much smaller, and on superficial examination they may indeed be overlooked; some cells at first sight merely look a little off-color, and it is seen only on very careful examination that the apparent polychromasia is in reality due to the presence of large numbers of minute dots. Very often, in especially anemic cells, the granules are arranged in the peripheral portion of the cell. Their number is exceedingly variable; generally speaking, it depends upon their size; when they are especially large they are relatively less numerous.

The granules may occur in cells of normal size or color, in poikilocytes, and in nucleated red cells, both of the normoblastic and the megaloblastic type, especially the former. Not infrequently they are seen in cells which are markedly polychromatic, but, like Grawitz, I do not believe that granular degeneration represents a phase of polychromasia.

In disease they are most constant and numerous in pernicious anemia, in lead poisoning, and in malaria; they are less constant and less numerous in the leukemias, in pseudoleukemia, in the cachexias referable to septic infection, syphilis, carcinomatosis, and in the final stages of tuberculosis. In chlorosis and in the anemia of chronic nephritis they are absent; in two cases of v. Jaksch's anemia, in which nucleated red cells were quite numerous, I obtained negative results.

In pernicious anemia granule cells are frequently found in the interval and at a time when the blood picture is otherwise practically normal. I have seen them most numerous in a case in which blood crises occurred from time to time (see page 60); almost every normoblast contained granules; non-nucleated granule, cells were however, at the same time present in large numbers Late in the disease, or in aplastic pernicious anemia, granule cells in my experience may be absent.

In lead poisoning granule cells are practically found without exception, and may be encountered at a time when no clinical symptoms are manifest. The amount of lead which is necessary to call forth their appearance is quite small, and it is a common experience to meet with a small number after the administration of lead in medicinal doses. I have found them after the ingestion of only 0.5 gram given in divided doses in the course of forty-eight hours. In cases of lead poisoning they persist for a long time after exposure has ceased. In one case of double wrist- and ankle-drop I could still demonstrate granule cells after five months.

In malaria granule cells are also common. Plehn found them in Europeans after a short sojourn in the tropics, and looked upon the granules as spores of the malarial parasite.

In septic cases and in the cachexia of carcinomatosis they are not numerous; in a case of cancer of the stomach with only 27 per cent. of hemoglobin, which I recently observed, I found no granule cells.

In the early stages of phthisis granular degeneration is not seen, but it may occur later, when a general septicemia has supervened.

Takasu[1] notes the occurrence of granule cells in infants affected with beriberi, especially in the acute severe cases. The same apparently occurs in the adult.

As regards the significance of the granules, Engel, Ehrlich, and others have suggested that they are most likely products of karyorrhexis. Others maintain, and I think rightly so, that they are not of nuclear origin. They may be found at a time when not a single nucleated red cell is demonstrable in the blood and nucleated red cells may be seen in which no sign of karyorrhexis is manifest, while the body of the cell is studded with granules. They may be found in nucleated cells which are undergoing karyokinetic division. Unlike the nuclei of the erythroblasts, the granules have no affinity for methyl green, which is a specific nuclear dye. This can be shown very well by staining with methyl-green-pyronin, when granular products derived from nuclei are stained green, while the stippling in the same cell appears red. A few observers claim to have stained the granules with methyl green; this merely shows that their dyes were contaminated with methylene blue.

According to Grawitz and others granule cells are not commonly found in the bone-marrow even when they are numerous in the circulating blood; when they do occur, they are not more numerous than in the peripheral vessels. Grawitz hence regards their presence as an indication of a degenerative change in the hemoglobin, and speaks of the phenomenon as "granular degeneration." Others regard the bone-marrow as their place of formation. Nägeli[2] thus comes to the conclusion that they are formed in the bone-marrow, because they only appear in artificial lead intoxication, when this is continuously established, and disappear when larger doses are given. Preceding the death of the animal they are not found. Opposed to the peripheral formation of the granules and Grawitz's degeneration hypothesis is the occurrence of granule cells in the blood of embryos.

According to Pappenheim stippling is not found in erythroblasts in the bone-marrow under normal conditions, but only when there is excessive regeneration, as in the embryo, in pernicious hemolytic anemia, in myelophthisic neoplastic anemia, in myelogenous pseudoleukemia and lymphadenoid leukemia and lymphosarcomatosis of the bone-marrow. Schmauch has observed similar appearances in the blood of healthy cats, and Engel has described the occurrence of granule cells in the blood of early cat embryos. I have found granule cells in the blood of various animals and occasionally one meets with an isolated cell in apparently normal individuals.

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[1] Folia hæmat., vol. i, p. 501.

[2] Münch. med. Woch., 1904.

Whether or not the granule cells which Vaughan[1] has demonstrated in normal wet specimens with Unna's polychrome methylene blue are identical with the variety described above is not certain. Their number varied quite constantly between 1.8 and 5 per cent. The examinations were conducted with wet blood, a drop of the staining fluid being placed upon the site of the puncture. At first the granules are red, but after some time they change through a purple to a pronounced bluish. Positive results were also obtained under various pathological conditions, especially in pernicious anemia, where their number was about ten times as great as in normal blood. In newborn infants they average 4.7 per cent. Vaughan regards the granules as nuclear remains and states that he rarely found stippling and nuclei in the same cell. In my own experience normoblasts in pernicious anemia are very frequently granular (see Plate III). Analogous results have been obtained by Cadwalader.[2]

Not to be confounded with "granular degeneration" is the stippling of Schüffner,[3] Ruge,[4] and Goldhorn, which is seen in many red cells infected with tertian parasites. This is brought out with methylene azure and may also hide the parasite from view.

LITERATURE.—E. Grawitz, "Ueber körnigeDegeneration d. rothen Blutzellen," Deutsch. med. Woch., 1899, No. 36, p. 585; "Klinische Bedeutung u. experiment. Erzeugung körniger Degenerationen," etc., Berlin. klin. Woch., 1900, p. 181; "Granular Degeneration of the Erythrocytes," etc., Amer. Jour. Med. Sci., 1900, vol. cxx, p. 277. Bloch, Deutsch. med. Woch., 1899, V. B. p. 279. Litten, ibid., No. 44. Behrendt, ibid., No. 44. White and Pepper, "Granular Degeneration of the Erythrocyte," Amer. Jour. Med. Sci., 1901, vol. cxxii, p. 266. C. E. Simon, International Clinics, 1902, vol. i, p. 69. Stengel, White, and Pepper, Amer. Jour. Med. Sci., 1902, vol. cxxiii, p. 873.

Cabot's Ring Bodies.—Cabot has drawn attention to the occasional occurrence in red cells of curious ring bodies which are usually stained red with Wright's modification of Leishman's stain, but which may also take on a blue côlor. He found such rings in pernicious anemia, in lead poisoning, and in lymphatic leukemia. I have been able to demonstrate the same structures with the eosinate of methylene blue, and could verify Cabot's observation that they occur in granule cells, but may also be found in apparently normal red corpuscles (Plate III). No doubt they bear some relation to the nucleoids. (See Fig. 15.)

LITERATURE.—Cabot, Jour. Med. Research, 1903, vol. ix.

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[1] Jour. med. Res., December, 1903.
[2] Amer. Jour., February, 1905, p. 213.
[3] Deutsch. Arch. f. klin. Med., 1899, vol. lxiv, p. 428.
[4] Zeit. f. Hyg. und Infectkrht., 1900, vol. xxxiii, p. 178.

Ehrlich's Hemoeglobinemic Innenkörper.—These structures may be encountered in red cells in conditions associated with extensive hemocytolysis the result of specific blood poisons. The individual body is round and characterized by its affinity for acid dyes.

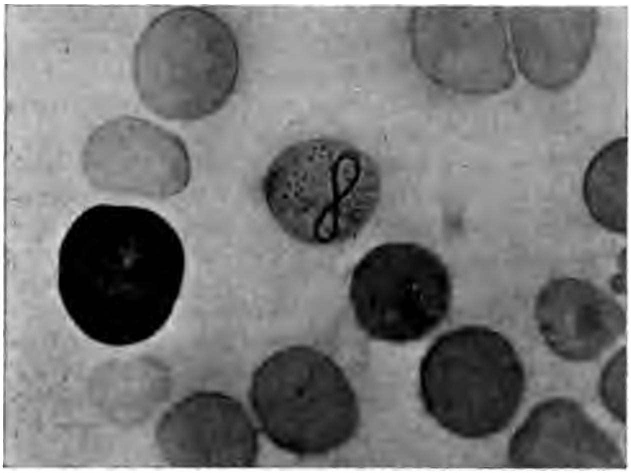

FIG. 15.—Cabot's ring bodies.

Nucleated Red Corpuscles. Erythroblasts.—Nucleated red corpuscles are not found in the circulating blood of normal individuals, excepting at birth and during the first days of life, when it is not unusual to meet with an occasional cell of this type. In the bone-marrow, however, they are always found. It is here possible to distinguish two types, viz., the normoblast and the megaloblast. The latter is ontogenetically the older and gives rise to the normoblast through a process of homoplastic differentiation by cell division; it thus bears the same relation to the normoblast which exists between the large lymphocyte and the small lymphocyte, and the amblychromatic myelocyte and the trachychromatic myelocyte (which see). The megaloblast itself results from the large lymphocyte through direct heteroplastic transformation and ages into the macrocyte, while the normoblast similarly develops into the normocyte. (See schema on p. 72.)

While at a certain period of embryonic life megaloblastic blood corpuscle formation plays a prominent role, megaloblasts are found only in small numbers in the bone-marrow of the normal adult. Normoblasts, on the other hand, are numerous and control the usual red corpuscle production exclusively.

The Normoblasts.—The normoblasts (see Plate III), like the normal red cells of the circulating blood, have a diameter which varies from 6 to 9 μ. The nucleus in the youngest cells occupies a central position, and is larger and relatively poorer in chromatin than in the older cells, where it is frequently located eccentrically. The size varies between 2 and 4 μ. The appearance of the normoblast in the peripheral circulation is variable (Plate III). In most cases young cells are seen with a radiary arrangement of the chromatin and polychromatophilic protoplasm. At other times older cells with densely staining pyknotic nuclei and oxyphilic protoplasm are encountered and again we may meet with cells in which manifest karyolysis is going on, as evidenced by budding of the nucleus and diminished chromatophilia. Fragmentation of the nucleus (karyorrhexis) may also be seen, as also free nuclei as such. Mitoses are not uncommon in pernicious anemia and leukemia.[1]

In the majority of cases in which normoblasts are found in the blood these are well preserved, but in myeloid leukemia more especially it is common to meet with cells in which the protoplasm surrounding the nucleus is much diminished in amount and presents a ragged outline. These cells are manifestly degenerating, and in many specimens the protoplasm will be seen reduced to a little hood which is attached to one side of the nucleus (Plate III). Such cells in my experience are always polychromatophilic and are apt to be mistaken by the beginner for lymphocytes.

The occurrence of normoblasts in the circulating blood is always evidence of stimulation of the bone-marrow, which may occur either indirectly, as the result of an "anemic" condition of the blood (secondary myelopathy), or directly, as in disease of the bone-marrow *per se* (primary myelopathy). We may accordingly meet with normoblasts in almost any form of anemia, be this the result of traumatism (posthemorrhagic), of inanition, or of organic disease.

In the acute forms of anemia they are apt to be most numerous, but even in the more chronic cases and in cachectic conditions specimens of blood may be obtained in which one or more normoblasts are seen in every field. In the secondary anemias, however, they are less common.

In active cases of pernicious anemia and the different leukemias normoblasts are quite constantly met with in fairly large numbers. Their continued absence in pernicious anemia is usually evidence of an aplastic condition of the bone-marrow and a bad omen.

At times there occur sudden invasions of the circulating blood by red cells, many of which are nucleated; this phenomenon v. Noorden terms a *blood crisis*, and it is noteworthy that the invasion of the red cells may be preceded and accompanied by a very extensive increase of the leukocytes. Ehrlich cites a case of hemorrhagic anemia, reported by v. Noorden, in which at the time of such a blood crisis the normoblasts were so numerous, while hyperleukocytosis of a high grade existed at the same time, that the blood condition strongly suggested the existence of a myeloid leukemia. The increase of the red cells in this case amounted to almost double their original number.

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[1] G. Dock, "Mitosis in Circulating Blood," Trans. Assoc. Amer. Phys., 1902, p. 484.

To estimate the extent of a blood crisis, the following examinations are necessary:

(*a*) A determination of the absolute number of red corpuscles.

(*b*) A determination of the ratio between the white and red cells.

(*c*) A determination of the ratio between the nucleated red and white cells.

EXAMPLE.—Supposing that in a given case 3,500,000 red corpuscles are found in the cbmm., while the ratio of the white to the red corpuscles is 1 to 100, and that of the nucleated red to the white 1 to 100; 3500 nucleated red corpuscles must hence be present in each cbmm. of blood—*i. e.*, 1 for each 1000 of normal red corpuscles.

The Megaloblasts.—These are usually from two to three times as large as the normoblasts, and may attain even more extensive proportions (Ehrlich's gigantoblasts). (See Plate III.) But some specimens are only a very little if at all larger than the common red cells; these probably represent young daughter cells. The megaloblasts are provided with a relatively large centrally located nucleus, which is wide-meshed and which with the triacid stain is not colored nearly so deeply as the normoblastic nucleus. In some specimens, indeed, the affinity for methyl green is so little marked that at first sight a nucleus can hardly be distinguished. With those staining mixtures, on the other hand, which contain methylene blue as base, it can always be fairly well made out. But owing to the fact that these cells are almost invariably polychromatophilic, the nucleus may at first be overlooked, as the ploychromatic protoplasm appears in the meshes of the nucleus and sometimes differs but little in color from the chromatin. The inexperienced not infrequently mistake such cells for large mononuclear leukocytes that are somewhat off-color; the character of the nucleus, however, viz., its wide meshwork, should prevent this mistake.

Mitoses in megaloblasts are at times seen.

As already mentioned the megaloblast is essentially a cell of embryonic life. After birth, under normal conditions a few megaloblasts may be found in the blood of very young infants, and it is noteworthy that in the severe types of secondary anemia megaloblasts are far more apt to occur in children than in adults. But even then they are rare. In the bone-marrow of the adult they are present in very small numbers. According to Ehrlich, the presence of megaloblasts in the blood is evidence of a reversion of blood formation to the embryonic type and of grave prognostic import. He regarded their presence as indicative of essential pernicious anemia; and, as a matter of fact, they are here quite constantly met with and represent one of the most important features of the disease. They are rarely numerous, however, and there are cases in which they are absent[1] (aplastic anemia).

The modern tendency is to regard the appearance of megaloblasts in the blood as evidence of an anemia of unusual severity, viz., as a degenerative-regenerative symptom, and not as an indication of any one disease. While they are undoubtedly most constant in pernicious anemia, they may also be met with in other forms. They have been found in leukemia, in the pseudoleukemia of infants, in lead poisoning, and, even in chlorosis, and as I have pointed out already, in some of the severe types of secondary anemia occurring in young children. In cancer of the stomach, according to Osler and McCrae, they are rarely if ever found. Askanazy[2] has reported an interesting case of bothriocephalus infection in which the megaloblastic type of blood regeneration disappeared after expulsion of the parasites—sixty-seven in number—and was replaced by the normoblastic type, the case ending in recovery.

The appearance of megaloblasts in extra-uterine life merely indicates an incomplete maturation of young elements, their consumption and consequent increased production. The following sketch, taken from Pappenheim, gives an idea of the relation of normoblasts and megaloblasts to the different types of anemia:

Under normal conditions Pappenheim's large lymphocyte (see schema, p. 72) gives rise to the young megaloblast, which in turn differentiates itself at once into young normoblasts. The young normoblast ages to the pyknotic normoblast and loses its basophilic nuclein as a result of chemical karyolysis. In this manner an apparently non-nucleated erythrocyte results, which loses its nucleoid later, in the blood, as blood platelet in consequence of variations in the tonicity of the plasma. In severe toxogenic anemias, on the other hand, there is an arrest of development upon an embryonic basis. A certain proportion of young megaloblasts multiplies homoplastically; another portion matures to old megaloblasts, while a third fraction only becomes differentiated to young normoblasts. Of these in turn one portion matures to the old forms, which dislodge their nuclei in the anemic serum in toto, while another portion loses the nucleus during the process of hastened maturation by karyorrhexis. As a consequence many of the anemic normocytes contain no nucleoids, and the blood as a consequence contains only small numbers of blood platelets.

Pyknotic normoblasts, as also young megaloblasts (of the type of the large lymphocyte), may thus be encountered in all forms of severe anemia of whatever origin. In the kryptogenetic type of pernicious anemia and bothriocephalus anemia, however, *old* megaloblasts (of the type of the large mononuclear leukocyte) are further seen, as also young normoblasts (of the type of the small lymphocyte) undergoing karyorrhexis.

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[1] Pane, "Sull' anemia progressiva mortale senza corpuscoli rossi, nucleati nel sangue," Riform. med., 1900, No. 263.

[2] Zeit. f. klin. Med., 1895, vol. xxvii.

Generally speaking the number of erythroblasts is no indication of the severity of the case, but merely indicates the extent to which the bone-marrow responds to the blood destruction. The appearance of megaloblasts is hence not necessarily an absolutely unfavorable symptom, but simply the expression of an unusually high activity of the erythropoietic tissue.

In cases of traumatic anemia unusually small nucleated red cells have at times been observed. These are termed *microblasts*. They have attracted but little attention and are quite rare. I have seen such cells, measuring not more than 3 to 3.5 μ, in a case of pernicious anemia at the time of the blood crisis, when large numbers of normoblasts were also present.

The Leukocytes.

General Characteristics.—The leukocytes, or white corpuscles of the blood, as seen in the wet preparation (Plate II), are roundish or irregularly shaped cells, which very in size, but for the most part are larger than the red corpuscles. They are all nucleated, and, as the term indicates, devoid of coloring matter. In a general way they may be divided into two distinct classes, viz., those which are granular and those which are not granular.

The *granular cells* (granulocytes) are by far the most numerous, and are characterized by the fact that they are capable of active locomotion. Even without a warm stage it is almost always possible to observe this in the ordinary wet preparation. The moving cells at once attract attention by their irregular outline. On careful examination with a high power it will be noted that the cell advances in a definite manner, which is quite analogous to what is seen in the ameba. The protoplasmic portion manifestly consists of two parts, viz., a non-granular hyaline ectosarc and a granular endosarc. As the leukocyte progresses the hyaline ectosarc advances with a flowing motion, forming a distinct layer in front of the granular endosarc, which itself then merges into the non-granular portion. The moving leukocyte is roughly pear-shaped, with the base in advance, while the rear end tapers markedly and frequently seems to drag behind it a small, roundish mass which, like the main body of the cell, is also granular. These granular leukocytes are true *phagocytes* and take up foreign matter into their interior like amebas. According to Metchnikoff, the phagocytic function is the most important function of the leukocytes, and the outcome of a bacterial invasion, figuratively speaking, will depend upon the superiority of the organisms engaged in warfare.

The nucleus of the granular leukocytes is either polymorphous—*i. e.*, it is composed of different lobes which are joined together—or it may be multiple. Such cells are hence spoken of as polymorphonuclear and polynuclear leukocytes, respectively. The polymorphous cells represent an earlier stage in the development of the polynuclear cell.

While the granules in the majority of the leukocytes are fine (Plate II), on careful search some cells will be found in which they are coarse and highly refractive. This coarsely granular variety is very characteristic in appearance and at once attracts attention. The cells are far less numerous, however, and, as a matter of fact, represent only from 1 to 4 per cent. of the total number of the leukocytes, while the finely granular variety represents from 60 to 70 per cent.

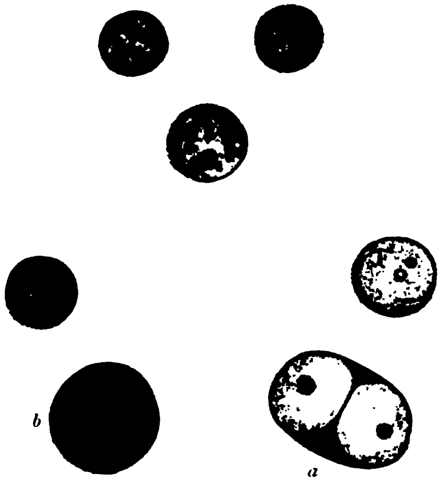
The *non-granular leukocytes*, in contradistinction to the granular variety, are mononuclear, with very little tendency to polymorphism. They are quite hyaline in appearance, and are readily overlooked by the beginner unless a somewhat subdued light is used in the examination. Two varieties may be recognized: one about the size of a red corpuscle, the other somewhat larger. The nucleus in both varieties occupies a considerable portion of the cell and is surrounded by a layer of protoplasm which is practically hyaline. Every cell, it is true, contains a few granules collected at a certain point along the periphery, where the protoplasm is more extensively developed than elsewhere; but these granules, in contradistinction to those which we see in the polynuclear varieties, probably represent nodal points in the cytoreticulum, and not a specific secretory product, as which Ehrlich and his school view the granules of the polynuclear variety. In the small mononuclear form one or sometimes two small, brownish granules can usually be discerned somewhere in the peripheral layer of the protoplasm. Of the significance of this granule, so far as I am aware, nothing is known, nor has its presence been previously described (Plate II).

The non-granular mononuclaer leukocytes, in contradistinction to the polynuclear granular variety, were formerly regarded as non-motile. Jolly, Wolff, and others have shown, however, that they also are capable of changing their form even though progressive locomotion may not occur. The change in form can readily be demonstrated even without a warm stage, and it will be observed that the nucleus takes an active part in these changes.

Classification.—While it is possible to distinguish the different varieties of leukocytes in the wet and unstained preparation, a more complete picture of the structure of the individual forms may be obtained from a study of stained preparations. The study of such preparations, moreover, forms the most satisfactory basis for the classification of the different forms. We distinguish the following varieties:

1. **The Lymphocytes (Small Mononuclaer Leukocytes, or Microlymphocytes)** (Plate IV).—The lymphocytes which occur normally

PLATE IV.



Lymphocytes.

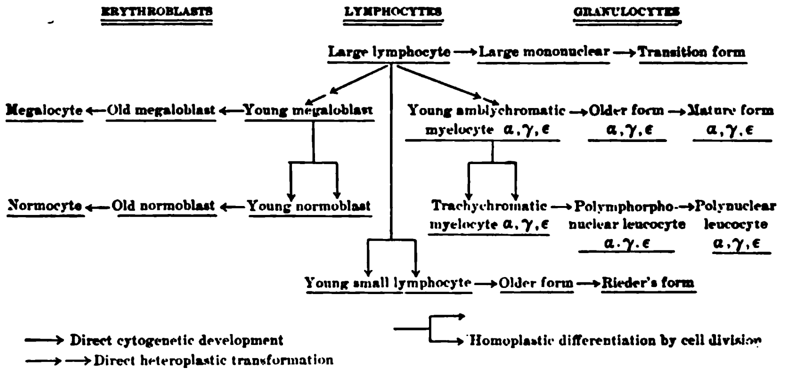
The cell *a* shows nucleus after division, each with a nucleolus; *b*, a plasma cell (irritation form, phlogocyte).

in the blood are for the most part a little smaller than the red corpuscles or of equal size. The nucleus is single and surrounded by a narrow rim of protoplasm which is generally described as non-granular; but, as I have pointed out, a few granules can almost always be made out in the wet preparation at a certain point along the periphery, where the protoplasm is a little more extensively developed. These granules, however, probably represent nodal points of the cytoreticulum, and are not to be regarded as in any way analogous to the granules which are met with in the polynuclear leukocytes. Nucleus and protoplasm are both basophilic, and, generally speaking, the protoplasm is so more markedly than the nuclus. This is best seen in specimens that have been stained with a methylene-blue mixture, where the lymphocytes for the most part present a comparatively feebly staining nucleus which is surrounded by a rim of dark blue. Other cells belonging to the same group, however, will also be seen in which this is not marked, but in which the staining affinities of both nucleus and protoplasm appear about the same or in which the protoplasm may even be lighter in color. These cells are generally a little larger than the first variety, with a somewhat broader zone of protoplasm and an eccentric position of the nucleus. They represent a later stage in the development of the deeply staining cell, and are sometimes termed medium-sized lymphocytes. A still larger form may also be met with, but is rarely seen under normal conditions. The staining properties of these *large lymphocytes* (*macrolymphocytes*) are essentially the same as those of the smaller varieties. The position of the nucleus may be either concentric or eccentric, as in the smaller forms, and a nucleolus is frequently demonstrable. This large type is notably seen in acute lymphatic leukemia, where it is usually the predominating cell. In smaller numbers it is also found under other pathological conditions which are associated with a hyperplasia of the lymphadenoid tissue.

According to Pappenheim, the large lymphocyte represents the ancestral cell (Ur or Stammzelle), from which all other leukocytes, as well as the red cells, are indirectly derived as the result of heteroplastic differentiation. (See schema, p. 72.) The large lymphocytes are identical with Benda's lymphogonia, Troje's lymphoid marrow cells, Nägeli's myeloblasts and the undifferentiated lymphoid cell of Michaelis, Wolff, and Türck.

With certain dyes, like methylene blue, the protoplasm of the lymphocytes does not appear perfectly homogeneous, but presents a peculiar granular appearance. This is referable to nodal points of the cytoreticulum and does not represent a true granulation. With methyl green, and hence with Ehrlich's triacid stain, the protoplasm is perfectly homogeneous and appears as a pale rim about the somewhat more deeply staining nucleus. While it is thus impossible with the usual dyes to demonstrate the existence of a true granulation in the lymphocytes, Michaelis[1] has called attention to the fact that with eosin-methylene-azure solutions (p. 132) distinct granules can be seen (azurophilic granules). Their significance, however, has not been established. Very curiously these granules could not be demonstrated in the lymphocytes obtained from the lymph glands directly, and it appears that they are present in only a certain percentage of those occurring in the blood. The number of granules in a cell is variable; in some only two or three are seen, while in others the protoplasm is literally studded with them. Their size varies between that of the common neutrophilic and that of the eosinophilic varieties (Plate V).

INTERRELATION OF LEUCOCYTES AND ERYTHROCYTES



In wet specimens, as I have pointed out, one or two reddish-brown granules are quite commonly seen in most of the lymphocytes. In stained preparations these cannot be demonstrated.

The outline of the cell in the smaller forms is usually fairly smooth, but in the larger varieties it is often shaggy, and at times specimens are seen with a number of distinct knobs.

The nucleus, in the smaller forms especially, is concentrically located, while in the larger varieties, in which the protoplasm is more extensively developed, it commonly occupies an eccentric position. In the stained specimens, especially in the larger cells, it is sometmies surrounded by a faint areola, which is probably owing to artificial retraction. The nucleus is more commonly oval or bean-shaped than round; deep invaginations are not often seen and fragmentation of the nucleus is rare. Such cells present an appearance which is altogether different from that of the true polynuclear elements.

Lymphocytes undergoing mitosis are sometimes seen in the blood of lymphatic leukemia. Characteristic figures, however, are comparatively rare, and it is more common to meet with cells in which

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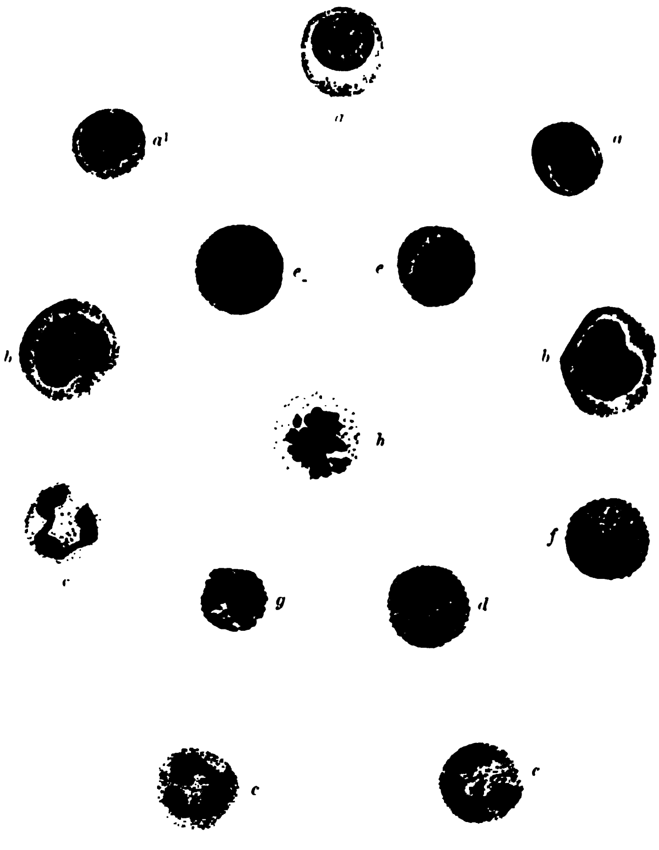
[1] Michaelis and Wolff, Virchow's Archiv, 1902, vol. clxvii, p. 151.

PLATE V.



Leukocytes.

a, small lymphocytes; *a'*, same, showing azurophilic granules; *b*, large mononuclear leukocytes; *c*, neutrophilic polymorphonuclear elements; *d*, adult eosinophile; *e*, neutrophilic myelocytes; *f*, eosinophilic myelocyte; *g*, mast-cell; *h*, karyokinetic normoblast. Stained with Wright's stain.

division of the nucleus has already occurred (Plate IV). In hematoxylin-eosin specimens it is usually possible to demonstrate a nucleolus, but in eosin-methylene-blue preparations my experience has been that they are not usually seen in the lymphocytes of the normal blood, and seem to be comparatively infrequent also in the blood of lymphatic leukemia. Occasionally, however, specimens are met with in which they are distinct, and at the same time multiple; in such cases active cell division seems to take place in the circulating blood.

In adults the number of the lymphocytes normally varies between 20 and 30 per cent. Higher values are found in young children, especially during the first year of life, when the lymphocytes constitute from 50 to 60 per cent. of the total number. At birth, however, they are less numerous than in adult life, viz., only about 15 to 16 per cent. Later they increase and by the twelfth day it is usual to have from 40 to 50 per cent. After the fifth year adult values are normally the rule.

In disease the number of the lymphocytes may be increased or diminished, conditions which are spoken of respectively as *lymphocytosis* and *lymphopenia.*

While it was formerly supposed that the lymphocytes originate only in the lymph glands proper, there is evidence that they may be formed wherever there is lymphoid tissue, and hence also in the spleen and in the bone-marrow. They are probably derived from the large lymphocytes of the germinal centres indirectly through a process of differentiating karyokinesis, and represent fully differentiated cells which are incapable of further development.

2. **The Large Mononuclear Leukocytes (Splenocytes).**—These are mostly two or three times as large as the red corpuscles and provided with a large single nucleus, which is surrounded by a relatively wide zone of non-granular protoplasm (Plate V). The nucleus in some cells is oval or elliptical, while in others it is more or less invaginated (Ehrlich's transition forms).

In the wet preparation the large mononuclear leukocytes are exceedingly hyaline, so that they are readily overlooked by the beginner. Both nucleus and protoplasm are basophilic, but much less markedly so than in the lymphocytes, and it is noteworthy that the protoplasm usually possesses a less marked affinity for the basic dye than the nucleus. Cells are also met with, however, in which the affinity for the dye is about the same in both. If by chance this occurs in specimens which are somewhat smaller than usual, a certain amount of difficulty arises in differentiating such small "large" mononuclear leukocytes from the older lymphocytes. A hard-and-fast line of distinction cannot here be drawn, and in every differential leukocyte count the personal equation will of necessity enter into consideration. The salient characteristics of the two types should, however, be borne in mind; in the lymphocytes the protoplasm is but feebly developed in relation to the size of the nucleus, while in the large mononuclear leukocyte the reverse is true. The protoplasm in the latter, moreover, is apparently much more delicate in structure, and is readily wrinkled by contact with adjacent cells; not infrequently cells of this type are found which have manifestly been torn or otherwise injured during the preparation of the specimen; the lymphocytes, on the other hand, are usually well-preserved and clear-cut, sharply defined cells.

In preparations that have been stained with Ehrlich's triacid both nucleus and protoplasm are very faintly colored and the latter appears perfectly homogeneous; but in specimens which have been stained with mixtures containing methylene blue as the basic component, the protoplasm presents a somewhat granular appearance, which, as in the lymphocytes, is referable to the existence of a cytoreticulum. A certain proportion of the large mononuclear leukocytes (including the transition forms), as in the case of the lymphocytes, also contain azurophilic granules.

Inclusive of the transition forms the large mononuclear leukocytes normally represent from 1 to 6 per cent. of the total number. They are relatively more numerous in young children, in whom the highest values are found between the sixth and ninth day after birth. Many of the cells at this time are of the type of the transition form; they may number 18 per cent.; but even in older children one commonly finds a larger proportion of these cells than in adults.

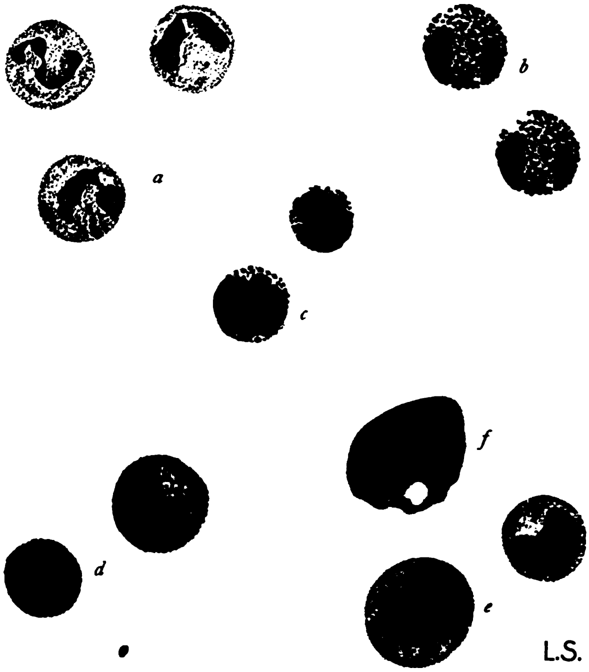
According to Pappenheim the large mononuclear leukocytes develop directly, cytogenetically, from the "large" lymphocytes, and then age into the "transition forms" which represent the final stage in the development of this type. The former view, according to which the large mononuclear leukocyte develops directly cytogenetically from the small lymphocyte and later ages into the polynuclear neutrophile, has been abandoned.

For the most part the large mononuclear leukocytes develop in the spleen (hence the term splenocytes).

3. **The Neutrophilic Polynuclear Leukocytes** (Plate VI).—These cells are a little smaller than the large mononuclear leukocytes and represent the finely granular variety already mentioned. They are active phagocytes and as such capable of progressive locomotion. The nucleus in the younger cells is polymorphous, while the older cells are actually polynuclear, the number of lobes varying from two to six. In stained specimens the nucleus shows a coarsely reticular structure with nodal thickenings and is very markedly basophilic. The protoplasm, on the other hand, is very feebly oxyphilic.

Embedded in the protoplasm are numerous fine granules—the ε-granulation of Ehrlich—which are characterized by their affinity for neutral dyes. Hence the term polynuclear *neutrophilic* leukocytes.

PLATE VI.



Granulocytes.

a, polynuclear neutrophilic leukocytes; *b*, polynuclear eosinophilic leukocytes; *c*, mast-cells; *d*, young eosinophilic myelocytes; *e*, neutrophilic myelocytes; *f*, the nucleus here has just undergone division; the clear space is a vacuole.

These granules are ordinarily very abundant; but in disease they may diminish in number until very few are left, and in some cases they may indeed be absent. Ewing[1] has called especial attention to the decrease in the number of the granules in the acute leukocytosis. I have observed total absence of granules in a case of trichinosis at a time when marked eosinophilia existed. Kast mentions an instance of general carcinomatosis with a leukocytosis of 120,000 in which 1.68 per cent. of the cells contained no granules. Hirschfeld describes the same occurrence in connection with growths involving the bone-marrow, and others have noted it in myeloid leukemia, where toward the end, in chronic cases, it is a fairly common phenomenon.

Associated with the diminution in the number of the granules there are frequently also degenerative changes affecting the nuclei. These may be of the type of karyolysis with swelling and loss of chromatin, or of karyorrhexis with hyperchromatosis and fragmentation of the nucleus. The former is the more usual in the acute leukocytoses, while the latter is seen especially in leukemia. In cases of the myeloid variety it is quite common to note complete fragmentation of the nucleus into six to ten segments. This phenomenon was first observed by Ehrlich in a case of hemorrhagic smallpox, and is of common occurrence in fresh exudates. Cell degeneration associated with loss of chromatin and swelling, while it no doubt occurs to a greater degree in disease, may also be observed under normal conditions.. In every dried and stained specimen a certain number of such cells will be found in which the nucleus appears as a much swollen and but faintly staining shadow, the *Kernschatten* of the Germans, sometimes surrounded by some of the granules, which appear scattered as though the cell had been burst asunder by force; at other times the Kernschatten alone remains and nothing is seen of the body of the cell.

I have stated that the loss of granules on the part of these cells may go on to a point where they are absent altogether. It may happen, however, that the granules are only apparently absent, and merely do not react as usual with ordinary dyes. A proper explanation of this peculiar behavior cannot be given, but every worker in blood is no doubt familiar with the phenomenon. Sometimes a change in the mode of fixation will cause the granulation to appear; at other times it may be demonstrated by the aid of some other dye.

Vacuolization of the polynuclear leukocytes is very much less common than in the case of the mononuclear elements.

While the neutrophilic leukocytes as a general rule are large cells, unusually small specimens are seen in the blood of myeloid leukemia. These dwarf forms must not be mistaken for the small cells which one may find in any specimen of blood where it is thick and where the process of drying has occurred slowly. In cells of this latter order the staining of the granules is also frequently deficient or they may not show at all.

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[1] Clinical Pathology of the Blood, Lea Bros., 1st ed., p. 113

Neusser[1] some years ago called attention to the fact that with a certain modification of Ehrlich's triacid stain it is possible to demonstrate the presence of basophilic granules about the nucleus of some of the polynuclear leukocytes, as well as the mononuclear elements. He, as well as Kolisch,[2] regarded the presence of these *perinuclear* granules as characteristic of the so-called uric acid diathesis. As tubercular disease, moreover, is usually not seen in such cases, Neusser thought the presence of these granules in cases of phthisis to be a favorable symptom. Futcher,[3] on the other hand, was unable to confirm these observations, and my own investigations[4] are likewise opposed to Neusser's conclusions. I was able to demonstrate the granules both in health and disease in almost every case, and was at one time even led to think that their absence was of more significance than their presence. A relation between their presence and the elimination of uric acid or xanthin bases certainly does not exist. Within recent years the subject has received no further attention, especially since Ehrlich expressed the belief that the granules are artefacts. He states that they are only exceptionally seen when solutions of chemically pure crystalline dyes are used, from the Actiengesellschaft für Anilinfarbstoffe in Berlin.

The polynuclear neutrophilic leukocytes are derived from corresponding mononuclaer forms—the neutrophilic myelocytes—which are normally found only in the bone-marrow. They result from these directly and represent their adult form.

Arneth[5] divides the polynuclear neutrophiles into five classes according to the number of nuclear lobes. Under normal conditions the percentage numbers of the different varieties remain fairly constant for one and the same individual, but vary somewhat in different people. The first class is represented by mononuclear forms and is subdivided into (*a*) mononuclear forms, corresponding to and identical with Ehrlich's *myelocytes* (see below); (*b*) forms with but slightly indented nuclei, the invagination not extending to a greater depth than the middle of the nucleus (the *metamyelocytes*); (*c*) cells in which the invagination extends farther than in form (*b*), but in which no separation into isolated loops or lobes has as yet occurred—the true *polymorphonuclear* variety. The two first varieties are essentially only seen under abnormal conditions, although an occasional metamyelocyte may at times be encountered in health. Cells of type (*c*) are present to the extent of 4 to 9 per cent. The second class comprises cells with two distinct nuclear fragments, which may appear either as two loops or two lobes. They constitute from 21 to 47 per cent.; the number, as already stated, varies somewhat with the individual, but is quite constant for one and the same person. In this class the cells with two loops normally always exceed those with one loop and one lobe, while true bilobes are rare. The third class presents three nuclear divisions and can be subdivided into four groups in accordance with the number of loops or lobes (see p. 81). Cells with two lobes and one loop approximate those with two loops and one lobe, while cells with three loops or three lobes respectively are in the minority. Conjointly the groups of the third class represent 33 to 48 per cent. Their number thus about equals that of group two, but has a tendency to be somewhat in advance. The fourth class is provided with four nuclear divisions with five subgroups and numbers 9 to 23 per cent. The fifth class finally comprises cells with five or more nuclear subdivisions and may be subdivided according to the same principle. Only 2 to 4 per cent. of the neutrophiles normally belong to this order. The various classes as just described represent different stages in the development of the neutrophilic cells, the myelocytes on the one hand being the youngest, and the polynuclear leukocytes with many lobes the oldest.

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[1] Wien. klin. Woch., 1894, p. 71. [2] Ibid., 1895, p. 797.

[3] Johns Hopkins Hosp. Bull., May, 1897.

[4] Amer. Jour. Med. Sci., vol. cxvii, p. 139.

[5] Die neutrophilen weissen Blutkörperchen. G. Fischer, Jena, 1904.

The polynuclear neutrophiles are the most common leukocytes of the blood and normally constitute from 60 to 70 per cent. of the total number. In young children they are relatively less numerous excepting during the first twenty-four hours of life, when they may number 73 per cent. But they rapidly diminish, so that values of from 20 to 40 per cent. may be regarded as normal during the first year. Low values continue practically to the twelfth year, though the numbers gradually rise. From the twelfth to the fourteenth year 60 per cent. may be regarded as an average; after that age the values given for the adult hold good.

4. **The Polynuclear Oxyphilic or Eosinophilic Leukocytes** (Plate VI). —In size and general appearance these cells resemble the polynuclear neutrophiles, and, like these, are capable of progressive locomotion. The granules—the α-granulation of Ehrlich—however, are much larger and highly refractive, and possess a marked affinity for acid dyes, such as acid fuchsin and eosin. Hence the term *oxyphilic* or *eosinophilic* leukocytes. With neutral dyes or basic dyes they will not stain. The appearance of the individual granules varies somewhat in stained preparations. Some are round, others oval; some appear to stain throughout, others make the impression of little vesicles with a limiting membrane, which alone takes the dye, while the interior remains unstained. This bleb-like appearance of the granule is one of the most marked characteristics. Barker[1]

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[1] Johns Hopkins Hosp. Bull., 1894, p. 93.

has shown that the granules contain iron. They are insoluble in ether and cannot be stained with osmic acid. They are therefore not composed of fat.

The protoplasm of the eosinophilic leukocytes is usually almost altogether hidden from view, owing to the dense packing of the granules; it is slightly basophilic. The nucleus is mostly bilobed, sometimes trilobed, and in stained specimens it is quite common to find the individual lobes unconnected by threads of chromatin; often the two lobes are situated at opposite poles. As a rule the nucleus is less markedly basophilic than that of the neutrophilic variety. A nucleolus is not seen.

The same degenerative changes which have been described in connection with the polynuclear neutrophiles may also be observed in the eosinophiles, and here, as there, one can at times note a material diminution in the number of the granules. I have never observed their entire absence, however, and it is noteworthy that in those cases of chronic leukemia in which the neutrophilic granulation may disappear the eosinophilic variety remains.

While the common eosinophile is a large cell, unusually small eosinophiles are frequently seen in the blood of myeloid leukemia. These should not be confounded with the small forms which may be seen in the thicker portions of almost any normal specimen. and which latter owe their small size to a gradual contraction during the process of drying.

Under normal conditions the percentage of the eosinophiles varies between 1 and 4.

While repeated attempts have been made to connect the eosinophilic leukocytes of the blood cytogenetically with the neutrophilic variety, there is no satisfactory evidence to support this view. On the contrary, there are strong reasons for believing with Ehrlich that, analogous to the neutrophilic variety, the polynuclear eosinophiles are normally formed in the bone-marrow, and here only from mononuclear eosinophilic cells—the *eosinophilic myelocytes.*

5. **The Mast-cells (Polynuclear Basophilic Leukocytes)** (Plate VI).—The mast-cells which are normally found in the blood are approximately of the same size as the polynuclear neutrophiles and eosinophiles. In myeloid leukemia, however, in which they are especially numerous, the size is more variable; on the one hand, they may measure only 3.5 μ in diameter, while on the other they may attain a dimension of 22 μ. The nucleus is polymorphous; but the tendency to form individual lobes is far less marked than in the corresponding eosinophilic and neutrophilic elements. Quite commonly it is leaf-like and flat in appearance. Its affinity for basic dyes is quite feeble, so that it is often difficult in stained preparations to make out the boundary line between nucleus and protoplasm. It is almost always excentrically located and usually has a fairly uniform

diameter of 4 μ. In the smaller specimens the nucleus occupies almost the entire cell.

Embedded in the protoplasm lie granules of variable size—the γ granulation of Ehrlich—some of which are fully as large as or even larger than the eosinophilic granules, while others are much finer. They are characterized by their affinity for basic dyes and the fact that with certain ones they stain metachromatically, viz., in a color which is different from that of the dye itself, which latter must be simple and not compound. Tissue elements which will stain in this manner are spoken of as chromotropic elements. Only a limited number of dyes have metachromatic properties. The most notable ones are the violet basic dyes hexamethyl violet, cresyl violet, thionin, neutral violet, and amethyst violet; further, the blue dyes methylene azure, cresyl blue, and toluidin blue, and the red basic dyes pyronin, acridin red, neutral red, and saffranin. With the latter group the mast-cell granules are colored yellow, with most of the violet dyes red, and with cresyl-violet R (extra) almost a pure brown. Methyl green does not stain the mast-cell granules unless it is contaminated with methyl violet, and for this reason the granules remain colorless in specimens stained with Ehrlich's triacid stain. In specimens fixed by heat and stained with aqueous alum hematoxylin solution the ϰ-granules are also not demonstrable. They have been dissolved; but there remains a well-defined spongioplasm, upon which the granules were deposited.

The mast-cell granules are *absolutely* basophilic, viz., they can only be stained with basic dyes, and retain the basic dye on subsequent differentiation in acid media. They are capable, moreover, of taking up the basic dye from its acidified solutions, as in the case of Ehrlich's dahlia-acetic acid mixture.

The granules of the common mast-cells of normal blood are resistant to water, while in myeloid leukemia cells are met with the granules of which dissolve with great readiness. Their chemical nature is still a matter of dispute, but there is a tendency to associate the mast-cell with the formation of mucin. This presupposes the identity of the blood mast-cell with the common mast-cell of connective tissue. In the past this has been tacitly assumed, but Pappenheim more especially has called attention to the fact that the hematogenic mast-cell differs from the histogenic form, and that the two probably represent different species. Pappenheim inclines to the view, however, that the granulation of the hematogenous mast-cells is not a true morphological granulation, but merely chemically altered lymphocytic spongioplasm, or a transport substance which has been taken up and metabolized.

The number of mast-cells varies between 0.2 and 1.0 per cent. Ewing states that he constantly failed to find mast-cells in the better class of healthy subjects, while in hospital and dispensary cases with minor ailments they appeared to be more numerous. My own observations do not bear this out; in my experience they are invariably present in health irrespective of the general nutrition of the individual.

The origin of the mast-cells of the blood has not been definitely ascertained. Ehrlich supposed that they originated from the connective-tissue cells as the result of hypernutrition, while Harris suggests that they may be derived from the large mononuclear leukocytes. According to Pappenheim, the mast-cell originates in the bone-marrow from a granular mononuclear type which corresponds to the eosinophilic and neutrophilic myelocytes.

6. **The Myelocytes.**—The myelocytes are mononuclear granular cells, which are *normally* not found in the circulation, but are encountered only in the bone-marrow.

Generally speaking, they represent the juvenile forms of the polynuclear leukocytes of the blood, and we accordingly distinguish three varieties, viz., the neutrophilic, eosinophilic, and basophilic myelocytes. The two last-named varieties, according to our present ideas, age directly into the corresponding polynuclear forms—*i. e.*, they become the common eosinophiles and the mast-cell. In the case of the neutrophilic variety it appears that two types exist, a smaller and a larger form, which Pappenheim[1] designates respectively as the trachychromatic and the amblychromatic form. These are ontogenetically derived, the first from the last, but only the trachychromatic variety ages into the common polynuclear neutrophile of the circulating blood. The nucleus of the amblychromatic form as it matures likewise becomes polymorphous, but normally the cell remains an inhabitant of the bone-marrow even then.

As regards the origin of the myelocytes, I incline toward Pappenheim's view, according to which all three varieties result from the large lymphocytes through a process of heteroplastic differentiation.

(*a*) THE NEUTROPHILIC MYELOCYTES.—These, as I have stated, are of two kinds. The one type, the *amblychromatic myelocyte* of Pappenheim, is a large cell provided with a relatively large, centrally located, round nucleus which stains but feebly with basic dyes. This is surrounded by a comparatively narrow zone of basophilic protoplasm which contains very fine neutrophilic granules. As the cell matures the nucleus becomes more or less invaginated and ultimately distinctly polymorphous. The protoplasm at the same time becomes relatively more abundant. Pappenheim speaks of this type as the *heteroplastic promyelocyte*. Such cells differ markedly in size from the common polynuclear elements which result from the second type of myelocyte.

The second type, viz., the *trachychromatic myelocyte*, is a smaller cell, which is essentially characterized by the fact that its nucleus stains quite intensely with basic dyes. The protoplasm is faintly oxyphilic and the granulation rather coarser than in the amblychromatic variety. As this cell matures the protoplasm becomes relatively more abundant and the nucleus distinctly polymorphous; it then constitutes the common neutrophile of the circulating blood. Between these two extremes there are transition forms, in which the nucleus is still single, but already shows a marked tendency toward polymorphism. These cells do not occur in normal blood. They have been described especially by Arneth. Pappenheim terms them *metamyelocytes* or *proleukocytes* (see Fig. 16).

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[1] Virchow's Archiv, vols. clix and clx.

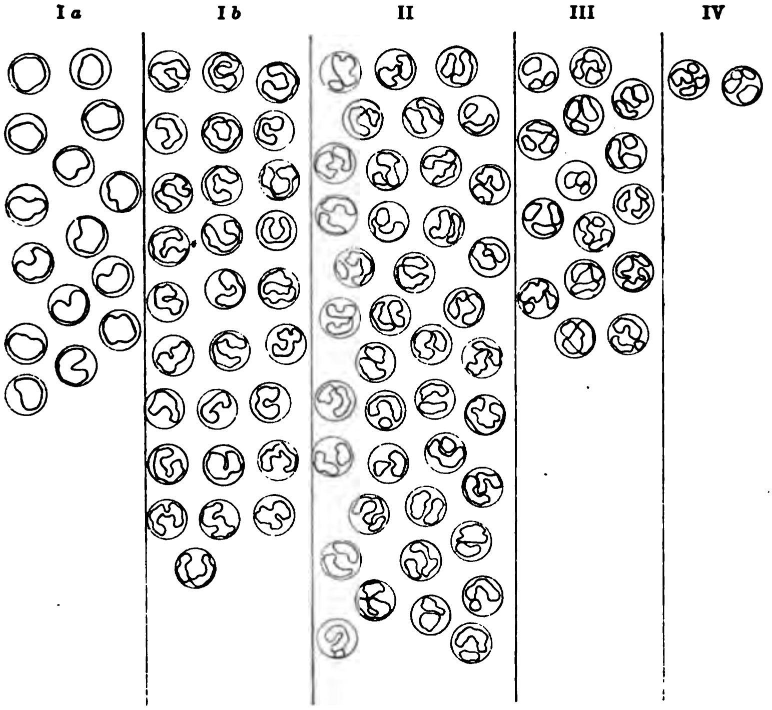

FIG. 16.—Karyolobism and polynucleosis of neutrophilic leukocytes.

Neutrophilic myelocytes undergoing mitosis are sometimes seen in the circulating blood in cases of myeloid leukemia; on the whole, however, they are rare, and it is more common to meet with cells in which the division of the nucleus has already taken place (Plate VI).

Müller and Jolly have shown that the neutrophilic myelocytes of the circulating blood are capable of active locomotion.

(*b*) THE EOSINOPHILIC MYELOCYTES.—In the more mature forms the color of the eosinophilic granulation on staining with eosin-methylene-blue mixtures is a pure eosin red. The younger forms, however, present a purplish-violet color, and some granules may indeed be a pure blue (Plate VI). This appearance is owing to the fact that the young eosinophilic granule is physically cyanophilic and chemically amphophilic, whereas the mature granule is physically erythrophilic, but chemically absolutely oxyphilic. This is well shown by staining such young cells with a mixture in which the basic dye is of a light color and the acid component dark, such as vesuvin on the one hand and water blue on the other. The mature eosinophilic granules will then take on the blue color of the water blue, while the young granules which stained blue with the eosin-methylene-blue mixture, and which we might accordingly have regarded as basophilic, are now likewise colored by the acid blue instead of the basic vesuvin, thus showing that they are in reality not basophilic, but amphophilic-cyanophilic

The protoplasm of the eosinophilic myelocytes is basophilic.

The size of the cells is quite variable; some are considerably larger than the corresponding polynuclear form, while others are much smaller. The cyanophilic cells are, generally speaking, the largest.

According to the observations of Müller and Jolly the eosinophilic myelocytes are capable of progressive locomotion.

(*c*) THE BASOPHILIC MYELOCYTES, like the eosinophilic and neutrophilic varieties, may be of variable size and are provided with a large centrically located nucleus, which is often distinguished only with difficulty from the surrounding protoplasm.

7. **Irritation Form** (STIMULATION FORMS, OR PHLOGOCYTES).—These are mononuclear non-granular cells, the protoplasm of which is stained a rich brown by the triacid mixture. The nucleus is round, eccentrically located, and colored a bluish green. Oftentimes it shows a distinct wheel-spoke structure. According to Türk, who first described these cells, they are met with under the same conditions as the myelocytes. Pappenheim regards them as plasma cells and as largely derived from histogenic lymphocytes as the result of a retrogressive degeneration, and characterized by hypertrophy of the cytoreticulum, increase of chromatin and chromatokinesis of the nucleus with coincident appearance of a markedly chromatophilic substance of exogenic origin. As intermediary cells Pappenheim regards lymphocytes without chromophilic protoplasm, but with radiary nuclei. It is thus essentially a pathological product. The cells have a spongioplastic cytoreticulum and vacuoles. They may attain a size of 30 μ. These cells, in my experience, are most frequently met with in the blood of children, where their number may attain 5 per cent. of all leukocytes. Wrench and Bryant[1] found this proportion in a girl of 10, in which, possibly as the result of gas poisoning, a severe anemia had developed.

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[1] Guy's Hosp. Repts., 1905, vol. lix, p. 333.

According to Pappenheim the occurrence of plasma cells in the blood is indicative of a chronic inflammatory process, either of the connective tissue or of the hemopoietic apparatus (tuberculosis, Hodgkin's disease, myeloma, etc.). I have found them relatively numerous in inflammatory conditions of the abdominal viscera (peritonitis, appendicitis, typhoid fever), and occasionally in measles.

The term irritation or stimulation forms indicates that the cells are found in connection with infectious, toxic, viz., inflammatory "irritation."

Iodophilia.—On staining blood smears of normal individuals with iodine (see p. 137) the protoplasm of the leukocytes is colored a bright yellow, while the nucleus is somewhat refractory and takes on a lighter tint. Under certain pathological conditions this staining quality is modified; cells are then seen in which reddish-brown granules appear in the protoplasm or it may occur that this presents a diffuse brownish color throughout. This intracellular reaction affects the polynuclear neutrophiles almost exclusively; the mononuclear elements *may,* however, also react, in which case one commonly sees large, pale-brown granules arranged about the nucleus in a single row. In eosinophiles the reaction does not occur. The extent to which the leukocytes are involved is quite variable; in some cases a few cells only are affected, while in others one is scarcely able to find a normal cell in an entire preparation.

An extracellular reaction also occurs, but is of little clinical interest, as it is not infrequent even in health; it occurs in small, roundish or oval masses, which are possibly true plaques, but which may also be small bits of protoplasm derived from leukocytes.

As to the nature of the substance which reacts with the iodine in the manner indicated, there is no uniformity of opinion. Ehrlich regards it as glycogen, and assumes that this is present normally in every cell in the form of a colorless compound, from which the free glycogen is under certain conditions split off, and can then be demonstrated as such. Czerny, on the other hand, looks upon the iodophilic substance as an antecedent of amyloid, while Goldberger and Weiss view it as peptone. Kaminer has shown that normal bone-marrow does not contain iodophilic leukocytes, but that they may here be found when they are present also in the blood. He concludes that the reaction is a degenerative phenomenon and not an evidence of regeneration.

The occurrence of the reaction in disease has been studied especially by Gabritschewsky, Czerny, Livierato, Kaminer, Cabot, and Locke. From these investigations it appears that septic conditions of all kinds may furnish a positive reaction, but that active suppuration may also occur without iodophilia (Reich, Küttner). Locke's list of diseases of this order includes general septicemia, abscesses (excepting in the earliest stages), appendicitis accompanied by abscess formation, general peritonitis, empyema, pneumonia, pyonephrosis, salpingitis with severe inflammation or abscess formation, tonsillitis, gonorrheal arthritis (in contra-indication to other forms), and acute intestinal obstruction where the bowel has become gangrenous. Locke concludes that no septic condition of any severity can exist without a positive reaction. In puerperal sepsis also it is said to be constant (Kaminer). In pneumonia with frank resolution it disappears in from twenty-four to forty-eight hours following crisis. In typhoid fever a positive reaction is not commonly obtained before the end of the second week, and it may indeed remain absent throughout the course of the disease. In the differential diagnosis between a serous and a purulent pleuritic effusion the absence of the reaction points to the former condition. Cerebral abscess may show the reaction, while in brain tumor it is absent (Gulland). In diphtheria it is only seen when there is much inflammation; it is never intense (Gulland).

In contradistinction to chlorosis, pseudoleukemia, and the common forms of secondary anemia of moderate intensity, iodophilic leukocytes are found only in the severer forms of anemia, such as pernicious anemia, leukemia (notably in acute cases), and the *severe* forms of secondary anemia.

In animals the reaction can be produced artificially by infection with the streptococcus, the staphylococcus, the Bacillus pyocyaneus, Löffler's bacillus, the anthrax bacillus, that of Friedländer, the Bacillus coli communis, or the typhoid bacillus; as also by means of ricin, abrin, and the diphtheria toxin. Following the injection of oil of turpentine, croton oil, mustard oil, and silver nitrate, the reaction may occur even though bacterial infection has been avoided. In man it is also said to occur following narcosis.

LITERATURE.—Ehrlich, Zeit. f. klin. Med., 1882, vol. vi. Gabritschewsky, Arch. f. exp. Path. u. Pharmak., 1891, vol. xxviii. Czerny, ibid., 1893, vol. xxxi. Goldberger u. Weiss, Wien. klin. Woch., 1897. Hofbauer, Centralbl. f. inn. Med., 1899. Livierato, Deutsch. Arch. f. klin. Med., 1894, vol. liii. Kaminer, Berl. klin. Woch., 1899, p. 119; and Deutsch. med. Woch., 1899, p. 206. Cabot and Locke, Jour. Med. Research, 1902, vol. vii. Locke, Boston Med. and Surg. Jour., 1902, p. 289. Reich, Beit. klin. Chir., xlii, 2. Küttner, Arch. f. klin. Chir., lxxiii, 2; and Centralbl. f. Chir., 1904, No. 27, Beil., pp. 3-5.

Leukocytosis.—While the number of red corpuscles is normally fairly constant, that of the leukocytes is subject to not inconsiderable variation. It is influenced by the age and sex of the individual, the process of digestion, menstruation, pregnancy, the bloodvessel from which the specimen is taken, etc. Generally speaking the number of the leukocytes varies between 3000 and 10,000, the exact number, *cæteris paribus*, depending upon the state of nutrition of the individual. In ill-nourished persons low values are the rule, while maximum numbers are generally associated with a state of exceptional vigor and good nutrition. These extreme figures, however, are uncommon, and as a general rule a count of 10,000 may be regarded as abnormal; 5000 to 6000 are the most common values which one finds if the examination is made with the individual in a fasting condition. During the process of digestion the figures are higher (see below).

An increase in the number of leukocytes is met with under the most diverse conditions, both in health and disease. When transitory, it is designated as *leukocytosis*. But it would be better to restrict this term to indicate the number of the leukocytes in a general way, and to speak of an increase as *hyperleukocytosis*, and of a decrease as *hypoleukocytosis*.

It will be convenient to consider the subject of leukocytosis under the following headings:

1*a*. Polynuclear neutrophilic hyperleukocytosis.
1*b*. Polynuclear neutrophilic hypoleukocytosis.
2*a*. Polynuclear eosinophilic hyperleukocytosis.
2*b*. Polynuclear eosinophilic hypoleukocytosis.
3*a*. Mast-cell hyperleukocytosis.
3*b*. Mast-cell hypoleukocytosis.
4*a*. Large mononuclear hyperleukocytosis.
4*b*. Large mononuclear hypoleukocytosis.
5*a*. Lymphocytosis.
5*b*. Lymphopenia.

The term *myelemia*, or *myelocytosis*, may be used to designate the appearance of myelocytes in the circulating blood, and in conformity with the three recognized forms we may speak of a neutrophilic, an eosinophilic, and a basophilic or mast-cell myelocytosis.

Until quite recently the general tendency in clinical laboratories has been to lay especial stress upon the absolute leukocyte count and to leave the relative values of the different forms out of sight. This should not be, and I cannot insist too strongly upon the importance of the relative count, which in many respects is far greater than a knowledge of the total number. For this reason also I have chosen the consideration of the subject of hyperleukocytosis on the basis of the classification just outlined.

Polynuclear Neutrophilic Hyperleukocytosis.—This is the most common form of hyperleukocytosis, and, as the term indicates, principally affects the polynuclear neutrophiles. Exceptionally it may be associated with a polynuclear eosinophilia, as well as with a lymphocytosis; but as a general rule both eosinophiles and lymphocytes are diminished. This diminution is often not only relative, but absolute as well. In very marked cases of hyperleukocytosis of this type it is not uncommon to meet with a few myelocytes which are then also of the neutrophilic variety; this is especially the case in children in whom the bone-marrow reacts more readily to stimulation. Eosinophilic myelocytes, on the other hand, are but rarely seen.

Clinically we must distinguish between an increase of the polynuclear neutrophiles which may occur in health and the common hyperleukocytosis which is observed in disease. We may accordingly speak of a physiological and a pathological form.

Physiological Hyperleukocytosis.—As physiological increase in the number of the leukocytes is notably observed at birth, during the process of digestion, in pregnancy, in association with severe muscular exercise, following the use of cold baths, etc.

Leukocytosis of the Newborn.—According to the experience of most observers, the number of leukocytes at birth varies between 10,000 and 23,000, of which over 70 per cent. are polynuclear neutrophiles. The number then falls and at the same time the lymphocytes increase. The curves of the two varieties cross between the sixth and the ninth day, and by the twelfth the lymphocytes are in excess. From the end of the first month to the fourteenth year there occurs a gradual increase of the neutrophiles and a decrease of the mononuclear elements. During the first year the total number of the leukocytes varies between 10,900 and 12,900; 9000 may be regarded as an average value from the first to the sixth year, and 7900 from then until the fifteenth year.

Digestive Leukocytosis.—The increase in the number of the leukocytes which is observed during the process of digestion affects both the polynuclear elements and the lymphocytes, though especially the latter. The eosinophiles are relatively at least diminished. The total increase rarely exceeds 3500 in normal adults, while in young children it may be much more marked. Schiff[1] cites an instance in which 19,500 leukocytes were counted one hour after birth, 27,625 after the first meal, and 36,000 after the fourth meal. It is especially pronounced after a preliminary period of fasting and following a meal rich in proteids. The maximum increase is usually observed between the third and fourth hour.

In cases in which a hyperleukocytosis exists from other causes, as in pregnancy, in inflammatory diseases, etc., digestive hyperleukocytosis does not occur. Lobenstine[2] in analyzing 20 cases of pregnancy in this direction found digestive leukocytosis in 13, no change in 1 and an actual decrease in 6. Apparently, however, he only made his examinations following the ordinary midday meal. In a few isolated instances it has also been found absent in apparently normal individuals without assignable cause. Under pathological conditions its absence is not uncommon, even though hyperleukocytosis referable to other factors may not exist. This is notably the case in carcinoma of the stomach, and it was once thought that the absence of digestive hyperleukocytosis in doubtful cases could be interpreted as evidence in favor of its existence.[1] Generally speaking, this is true even now, and we may say that in about 90 per cent. of all cases of carcinoma of the stomach digestive hyperleukocytosis does not occur. The symptom, however, is not pathognomonic, as a number of instances of carcinoma have been reported in which there was a distinct increase, and as digestive leukocytosis *may* also be absent in other conditions. In anemic individuals, from whatever cause, especially large amounts of proteids are sometimes necessary to elicit an increase of the leukocytes (Müller[2]) and in some cases a subnormal number may even be encountered (Rieder[3]).

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[1] Zeit. f. Heilk., vol. xi, p. 30, and 1890, p. 1.

[2] Amer. Jour. Med. Sci., August, 1904.

To study digestive hyperleukocytosis, it is well to proceed as follows:

(*a*) The first blood count should be made after the patient has fasted for about seventeen hours.

(*b*) After this period he receives a test meal consisting of from 200 to 1000 c.c. of milk and one or two eggs, the amount varying with the condition of the patient.

(*c*) Further blood counts are made one, two, three, and four hours later.

(*d*) The existence of a digestive hyperleukocytosis should only be regarded as proved if an increase of at least 1500 cells occurs, providing that maximal amounts of food have been taken. If smaller amounts have been given, an increase of 1000 cells is sufficient to establish its existence, provided that the same result is observed on repeated examination.

Leukocytosis of Pregnancy and Parturition.—The hyperleukocytosis which is observed in pregnancy is particularly marked during the last five months, and appears to occur quite constantly in primiparæ, while in multiparæ exceptions are common. In an analysis of 55 cases Hubbard and White[4] obtained positive results in 44—*i. e.*, in 80 per cent.—most marked and constant in young primiparæ. Rieder in an analysis of 31 cases noted a hyperleukocytosis in 20, all the negative cases being multiparæ. In a series of 17 multiparæ an increased number of leukocytes was noted in only 7. In Rieder's series the number of leukocytes varied between 10,000 and 16,000, with an average of 13,000. This represents the usual increase, but at times much larger numbers may be observed; Cabot thus reports 3 cases with a leukocytosis of from 25,000 to 37,000

Lobenstine gives the following figures as the result of an analysis of 50 cases in the ninth month:

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[1] Schneyer, "Das Verhalten d. Verdauungsleukocytose b. ulcus rotundum u. carcinoma ventriculi," Zeit. f. klin. Med., vol. xxvii, p. 219.

[2] Zeit. f. Heilk., 1890, p 213.

[3] Beit z. Kenntniss d. Leukocytose, 1892.

[4] Jour. of Exper. Med., 1898, p. 639.

Average count	10,600
Highest count	18,000
Lowest count	5,400
Average in primiparæ	9,346
Average in multiparæ	11,854

Absence of leukocytes in 7 cases.

During actual labor there is an increase of the leukocytes over and above the numbers previously observed in pregnancy; 30,000 cells may then be noted.

Lobenstine's figures in his series of 50 normal cases on the third day of the puerperal period are the following:

Average count	12,400
Highest count	20,400
Lowest count	5,600
Average in primiparæ	13,200
Average in multiparæ	11,600

No leukocytosis in 8 cases.

The highest numbers are met with in severe and protracted cases, especially after rupture of the waters. This form of hyperleukocytosis subsides after the expulsion of the child, and at the end of the first or second week normal values are again reached, though the gradual decline may be interrupted by a temporary increase now and then, referable to various minor disturbances during the puerperal state. In many cases normal values are reached much earlier, and by the third day, as a rule, the number is as low as it was before labor.

As in the case of the digestive leukocytosis, the hyperleukocytosis of pregnancy and the puerperal state is brought about by an increase both of the polynuclear neutrophiles and the lymphocytes, while the eosinophiles remain passive.

Leukocytosis following Baths, Muscular Exercise, etc.—The increase of the leukocytes following cold baths may, according to Thayer,[1] amount to nearly 300 per cent. In 20 cases of typhoid fever he found 7724 leukocytes on an average before and 13,170 after the usual Brand bath. In his own person, while in health, the leukocytes on one occasion numbered 3250 before the bath, while twenty minutes later they had increased to 12,500. Such an increase is, however, only observed after a bath of moderate duration, while a prolonged cold bath diminishes the number. Hot baths have exactly the opposite effect, viz., those of short duration produce a decrease, those of long duration an increase.

Violent muscular exercise, as well as massage, produces a temporary hyperleukocytosis.

Pathological Hyperleukocytosis. 1. *The Hyperleukocytosis of the Acute Infections.*—In the acute infectious diseases hyperleukocytosis referable to an increase of the polynuclear neutrophiles is the rule. It is seen in pneumonia, erysipelas, diphtheria, scarlatina, the various pyogenic infections in the narrower sense of the term, in parotitis, acute articular rheumatism, epidemic cerebrospinal meningitis, etc. Typhoid fever and measles represent notable exceptions if we disregard the very earliest stage in the development of the disease, when an acute hyperleukocytosis may also be observed (see p. 97).

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[1] Johns Hopkins Hosp. Bull., April, 1893.

Generally speaking, the increase in the number of the leukocytes in the acute infectious diseases is directly proportionate to the intensity of the infection and the power of resistance on the part of the individual. Where this is particularly feeble or the virulence of the infection is especially intense, an absolute increase of the total number of the leukocytes may not take place, although a relative increase of the polynuclear neutrophilic elements will probably always be observed. A recognition of this fact is of importance and serves to illustrate the special value of the *differential* count.

In *pneumonia* the increase in the number of the leukocytes is usually marked. On an average it amounts to about 24,000 cells above the normal (Cabot). In rare cases a leukocytosis of 100,000 and over has been observed. The hyperleukocytosis sets in quite early—within a few hours following the initial chill—and persists until the time of the crisis, when it rapidly disappears; the decrease may indeed precede the critical fall of the temperature. When the disease terminates by lysis the return to the normal is more gradual. A pseudocrisis is not accompanied by a fall in the number of the leukocytes. When resolution is delayed or complications occur, the hyperleukocytosis persists.

Absence of hyperleukocytosis, excepting in very mild cases, will usually warrant a fatal prognosis; exceptions, however, occur, and it is well in any case to base prognostic conclusions not upon a single count, but upon the result of repeated examinations, as it is not uncommon to meet with considerable fluctuations in the course of the disease. Sears and Larrabee[1] found the mortality much greater when the leukocytes numbered less than 10,000 than when they were more numerous; and, according to Löper, a progressive increase of the neutrophiles beyond 90 to 95 per cent. may be regarded as an unfavorable symptom irrespective of their total number. Associated with the increase of the polynuclear neutrophiles in pneumonia there is a relative diminution of the lymphocytes. The eosinophiles are greatly diminished; they may indeed be absent. Their return may occur before the beginning of the crisis and may be viewed as a favorable symptom.

In *bronchopneumonia* the total increase of the leukocytes is not so great as in the acute croupous form.

In *erysipelas*, as in pneumonia, the hyperleukocytosis is generally proportionate to the intensity of the morbid process and also terminates by crisis. The increase of the leukocytes beyond normal may amount to 15,000; in many cases, however, the total number does not rise much beyond the upper limit of the normal. At the height of the disease the eosinophiles are much diminished or absent.

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[1] Med. and Surg. Rep. of the Boston City Hosp., 1901, 12th series, Dec. 1st.

In *diphtheria* a well-marked increase is the rule. Generally the count does not exceed 25,000 to 30,000, but in fatal cases it is common to meet with larger numbers. Ewing[1] speaks of one case with lymphocytosis in which the count was 72,000, and cites a peculiar instance reported by Felsenthal[2] marked by hemorrhagic eruption in which 148,000 were counted. As Ewing suggests, this was probably an agonal hyperleukocytosis. As a rule, from 25,000 to 50,000 cells are met with in fatal cases. In children the general increase of the leukocytes is frequently associated with a relative lymphocytosis. The eosinophiles are diminished in number and may indeed be absent. It is interesting to note that excepting a temporary diminution immediately following the injection the leukocytosis is in no wise influenced by the antitoxin treatment. Besredka,[3] however, states that the grade of the polynuclear neutrophilic hyperleukocytosis after the administration of the serum indicates the prognosis. Thus, if one or two days after the injection the percentage of the neutrophiles is 60 or more, the prognosis is good; with a higher temperature and 50 per cent. it is bad, and with a lower percentage the disease is fatal. Simon[4] finds that the occurrence of hyperleukocytosis and hyperpolynucleosis four hours after the injection is a favorable sign. The exanthem which occasionally follows the injection of antidiphtheritic serum is accompanied by a polynuclear neutrophilic hyperleukocytosis.

In *tonsillitis* there is an increase of the leukocytes of approximately the same intensity as in diphtheria, with a similar diminution in the number of the eosinophiles.

In *septic conditions*, in general, hyperleukocytosis is of constant occurrence at some stage of the disease, unless the infection is very mild or very severe. Even in those cases in which the absolute increase of the leukocytes is not marked, or, as in certain very virulent cases absent altogether, *the neutrophiles are relatively increased and the eosinophiles coincidently very much diminished or absent altogether.* This association I have termed the *septic factor* and I cannot insist too strongly upon its value in the diagnosis of acute infections with the group of pyogenic organisms.

Especially important is the study of the leukocytosis in *appendicitis*. According to Curschmann's initial studies in this direction a leukocytosis of 22,000 is strongly suggestive of an existing abscess; if the count remains stationary at this point, or if it increases but once to 25,000, suppuration may be regarded as established. These results have been largely confirmed by other investigators. Exceptions, however, occur and surgeons perhaps not unnaturally decline to be guided in their operative work by the results of the blood count only. Personally I value the absolute count very highly in the study of the progress of an appendicitis, but only in so far as an increase, and, above all, a progressive increase is concerned. Normal figures or a decreasing leukocytosis are very dubious factors and should be viewed with reserve. In my estimation the differential count is much more valuable in the study of these cases and far less apt to mislead. A decreasing neutrophilic hyperleukocytosis with a return of the eosinophiles is a favorable symptom in all cases. A drop in the total count with a continuance of the relative polynucleosis, on the other hand, usually means added danger and only too often perforation.

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[1] The Blood, loc. cit.
[2] Arch. f. Kinderheilk., vol. xv, p. 78.
[3] Annal de l'Inst. Pasteur, 1898, vol. xii, 5, p. 305.
[4] Journ. de physiol. and pathol. gén., vol. v, p. 887.

I here append extracts from Bloodgood's paper,[1] which gives a good idea of the usual findings in appendiceal and related abdominal affections, but regret that the absolute counts only have been considered.

Observed within forty-eight hours the number of white blood cells is in the majority of instances of great value in pointing to the extent of the inflammatory condition of and about the appendix. Cases of recurrent appendicitis or of appendicitis suffering from the first attack, first observed practically at the end of the attack when the clinical symptoms are subsiding, rarely show an increase in the white cells. In a few instances, first observed within forty-eight hours after the beginning of the attack, but when the symptoms are subsiding, there have been a few leukocyte counts of 15,000, which have fallen rapidly within a few hours to 10,000 and 7000. In the cases admitted within forty-eight hours with acute symptoms, if on account of the clinical picture operation has been delayed, a falling leukocytosis has always been observed. These patients have recovered, and at a later operation the appendix was found to be the seat of a diffuse inflammation, but there was no evidence of pus outside the appendix. In one case admitted sixteen hours after the beginning of the attack the leukocytes fell in ten hours from 17,000 to 13,000, and in twenty-four hours to 11,000, associated with disappearance of the symptoms. With one exception, the highest first leukocyte count in this group has been 17,000, falling in a few hours to 12,000, 9000, or even lower. A patient admitted twenty hours after the beginning of the acute attack had a leukocytosis of 22,000; the clinical symptoms, however, were not very marked. The patient was observed eight hours; during this period the leukocytes fell to 16,000 and the local symptoms practically disappeared. Within the succeeding twenty-four hours the leukocytes were 11,000, then 8000, 7000, and 6000. Although this patient with a leukocytosis of 22,000 at the end of twenty hours, recovered, and there is every reason to believe that the inflammatory condition about the appendix subsided, nevertheless it is an exception to the general rule, and it would be safer, I believe, to operate in those cases of acute appendicitis observed within the first forty-eight hours with a leukocytosis of 20,000.

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[1] Blood Examination as an Aid to Surgical Diagnosis, Amer. Med., 1901, p. 306

In acute diffuse appendicitis with operation and recovery the highest count observed was 25,000 thirty-six hours after the beginning of the attack. At operation in this case intense inflammation and a large amount of exudate were found about the appendix.

In gangrenous appendicitis with operation and recovery the leukocytosis is higher (25,000 to 35,000) and rises more rapidly. As Bloodgood says, the study of the leukocytosis is here of the greatest importance in the early recognition of a grave inflammatory condition of the appendix, which without doubt would lead to general peritonitis and death unless early operation be instituted.

A very high leukocytosis within forty-eight hours after the beginning of the attack is suggestive, but not at all positive, of beginning *peritonitis*. The leukocyte count, however, does not seem to help in such cases with regard to prognosis. After the second day in cases in which the peritonitis has been present longer Bloodgood never has observed recovery with a low leukocyte count. If the leukocytosis remains still high at this period, however, the prognosis seems better for ultimate recovery after operation.

In chronic suppuration the results are less decisive; there are cases indeed in which notwithstanding the existence of extensive intraperitoneal accumulations of pus no increase of the leukocytes occurs.

In intestinal obstruction an increase of the leukocytes associated even with very slight symptoms is of the highest importance in the early recognition of the lesion. Bloodgood states that in a large group of cases the leukocyte count may rise to 20,000 within twelve hours after the beginning of the obstruction.[1] Within the first twelve to twenty-four hours a few observations would demonstrate that if the leukocyte count rise above 25,000 or 30,000, the probabilities are that one will find gangrene of the obstructed loops or beginning peritonitis. If observed on the second or third day after the beginning of the symptoms, it is difficult to make a differential diagnosis with regard to gangrene or peritonitis. After the third day, in cases in which there is no gangrene and no peritonitis, or in which the auto-intoxication is not yet very grave, the leukocytes still remain high—15,000 to 23,000—according to the degree of obstruction: complete, higher; partial, lower. In the presence of gangrene peritonitis or grave auto-infection, the leukocytes begin to fall. If the patient is admitted after the third or fourth day, with a history of intestinal obstruction, and still has a high leukocyte count, the prognosis is good for operation. If the count is low, and especially if it is below 10,000, the probabilities are that on operation extensive gangrenous peritonitis will be found; or the patient will be so depressed by auto-intoxication that reaction does not follow relief of the obstruction.

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[1] Cases have indeed been observed with an absolute count of 50,000, and more.

In *amebic liver abscess* there is a comparatively low leukocytosis, viz., 10,000 to 17,000, rarely over 20,000.

In *eclampsia* there is usually marked hyperleukocytosis, the degree *cœteris paribus* depending fairly closely upon the apparent toxicity of the case (16,000 to 20,000 in mild cases). With a good resistance the increase is especially marked (46,000 to 54,000). A sudden increase generally indicates an aggravation of the condition in an individual of good resistance (as high as 100,000). A low count in a highly toxic patient is of bad omen (19,000 dropping to 13,800 by the second day following delivery). A leukocytosis originally high that falls rapidly in a badly toxic patient is likewise a danger signal (100,000 to 45,200 in one day) (Lobenstine).

In the differential diagnosis between *ruptured tubal pregnancy* with associated severe internal hemorrhage and acute peritonitis a high leukocyte count speaks in favor of the first condition. In a slowly developing peritonitis, on the other hand, hyperleukocytosis may also be observed. With small hematoceles (referable to tubal pregnancy) the leukocytes may be normal.

In *pyosalpinx* high leukocyte counts are almost always seen.

In *uterine carcinoma* hyperleukocytosis is usually only seen when there is extensive ulceration. *Myomas* only lead to hyperleukocytosis as the result of extensive hemorrhages. In *hydrosalpinx* and *salpingo-oöphoritis* the same is seen. In connection with *ovarian cystoma* the leukocyte values are usually normal; if, however, peritoneal irritation exists (as manifested by sensitiveness to pressure and ascites), a marked increase may occur. In such cases in contradistinction to pus cases the iodine reaction is negative.

In *scarlatina* hyperleukocytosis is a constant feature of the disease.[1] It usually begins two or three days before the appearance of the rash; sometimes even as early as the sixth day. The acme is reached on the second or third day; on the fourth medium values are found. Then the decrease usually begins, although this is sometimes delayed until the eighth or ninth day; normal values are not reached until the end of the second or the beginning of the third week. In light cases the leukocytosis amounts to from 10,000 to 20,000 cells, in cases of moderate severity 20,000 to 30,000 are average figures, while in fatal cases 40,000 are common values. The hyperleukocytosis is scarcely influenced by the height of the temperature, the angina, the rash, desquamation, or complications, excepting that in the latter case its duration is influenced by the nature of the pathological process. The hyperleukocytosis is due to a large increase of the polynuclear neutrophiles, which may represent 94 per cent. of all leukocytes. The lymphocytes are proportionately diminished unless glandular complications occur, when they may reach maximum normal values. The eosinophiles in light and moderately severe cases are at first normal or subnormal, they then gradually increase and reach maximum values (8 to 15 per cent.) in the second or third week, after which they return to normal. In severe cases they diminish to zero.

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[1] Van der Berg, Arch. f. Kinderheilk., vol. xxv, p. 321. Mackie, Lancet, Aug. 24, 1901. Reckzeh, Zeit. f. klin. Med., 1902, vol. xliv, p. 201 (full literature).

In *acute articular rheumatism* the degree of hyperleukocytosis is proportionate to the severity of the attack. In McCrae's[1] analysis of 83 cases the average count was 11,776; in 29 it was below 10,000. Taking the average of the remaining 54 cases we have 14,260. In 17 the count was over 15,000 and in 4 over 20,000; the highest figure was 38,000. It is noteworthy that hyperleukocytosis was noted in all cases of complicating pericarditis in which a count was made, but that normal values were obtained in many cases of undoubted endocarditis. In pericarditis 15,000 to 19,000 were average values; 35,000 was the highest count noted. Generally speaking, when the number of leukocytes in acute articular rheumatism rises to 20,000 or higher, pericarditis or pneumonia may be suspected (Türk, Ewing). When the total increase of the leukocytes is only slight, the percentage values are not especially disturbed, but with a marked hyperleukocytosis the polynuclear neutrophiles are materially increased. The eosinophiles are commonly absent in the early stages of the disease, while later they are always present in moderate numbers, and after defervescence they are usually increased.

In *tubercular disease* hyperleukocytosis is observed only when secondary infection with pus organisms has taken place, while in pure cases the number remains normal. As the conditions for a secondary infection are more favorable in some parts of the body than in others, such as the lungs and kidneys, hyperleukocytosis is commonly present when these parts are involved. In the third stage of pulmonary tuberculosis there is usually a leukocytosis of from 15,000 to 20,000, which is referable to a well-marked increase of the polynuclear neutrophiles, while the eosinophiles are diminished. In the second stage, owing to a concentration of the blood no doubt, values ranging between 8000 and 10,000 are common, while in the first stage normal values are found.[2] In tubercular peritonitis the leukocytosis is variable. In 36 cases of 46 analyzed by Shattuck the number was below 10,000; where it is higher pus may or may not be present.

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[1] Jour. Amer. Med. Assoc., 1903, vol. xl, p. 210.

[2] Appelbaum, loc. cit., p. 61.

In the *epidemic form* of meningitis the count may range between 32,000 and 34,000 early in the disease. Later, when the temperature rarely rises above normal the count may drop to from 9000 to 10,000. In fatal cases the highest counts are usually seen (35,000 to 55,000), but it is to be noted that a high count does not necessarily imply a fatal ending. In the *tubercular form* hyperleukocytosis is also observed in a large percentage of cases, but is not apt to exceed 24,000; in 60 per cent. of Koplik's cases it was under 20,000. In many of these cases the hyperleukocytosis is due to a complicating terminal pneumonia.

In serous non-tubercular *pleurisy* the leukocytes are not increased, while in the tubercular cases the number may rise to 15,000 to 20,000. This increase, however, is probably greatly dependent upon the extent of the primary lesion.

In *empyema* there is marked hyperleukocytosis (22,000 to 29,000), which disappears after evacuation of the pus.

In *smallpox* a hyperleukocytosis is observed only in the severer cases and when pustulation takes place. In the milder form no increase occurs.

In *Malta fever* a marked increase of the polynuclear neutrophiles may occur just before the onset of the fever; later there is absence of hyperleukocytosis. In a case observed in the United States 11,564 leukocytes were counted, all varieties being present in normal proportion.[1]

In *bubonic plague* a moderate increase of the leukocytes is the rule; a few instances have been reported in which over 100,000 cells were counted, the increase being largely due to neutrophiles.

In uncomplicated cases of *typhoid fever*, during the first few days, there may be a leukocytosis of 3000 to 5000 beyond the normal, referable to an increase of polynuclear neutrophiles. Subsequently they diminish and a relative lymphocytosis comes to the foreground (see especially p. 97).

In *tetanus* a moderate neutrophilic increase occurs.

In *uncomplicated measles* there is in the beginning a moderate relative increase of the polynuclear neutrophiles, 76 to 82 per cent.; but this is not associated with an absolute increase of the leukocytes, but with a decrease. Later there is a relative decrease of the neutrophiles to 50 to 60 per cent., while the absolute number is increased.

According to Wilson and Chowning, a hyperleukocytosis of about 12,000 is usual in cases of the so-called *spotted fever* of the Rocky

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[1] Musser and Sailer, Phila. Med. Jour., 1898, p. 1408, and 1899, p 89.

Mountains.[1] But I have also seen the blood from several cases in which no increase existed.

In some cases of *enterogenous cyanosis* the number of leukocytes may be increased to 20,000, but as a rule it varies between 4000 and 10,000. The relative figures are scarcely affected.

In *dementia paralytica*, throughout the disease, there is an increase of the polynuclear neutrophiles which reaches its height during the terminal stage, while the eosinophiles are for the most part materially diminished. Paralytic attacks are accompanied by a further increase of the neutrophiles.[2]

2. *Anemic Hyperleukocytosis.*—Hyperleukocytosis referable to an increase of the polynuclear neutrophiles is observed in various forms of acute and chronic secondary anemia. It is especially marked after hemorrhages, when the number of leukocytes may increase to 30,000 and even more. Generally speaking, the degree of increase is proportionate to the amount of blood lost and the recuperative power of the individual. Rieder noted a leukocytosis of 15,000 after a pulmonary hemorrhage; 32,600 after a hemorrhage due to uterine cancer, and 26,500 after a hemorrhage referable to gastric ulcer.

If we except the myeloid type of leukemia, in which an absolute increase of the polynuclear neutrophiles is associated with a relative decrease, hyperleukocytosis is not met with in uncomplicated cases of the primary anemias.

3. *Cachectic Hyperleukocytosis.*—A cachectic hyperleukocytosis has been described in connection with malignant disease, phthisis, etc. Ewing states that in the majority of cases of tertiary syphilis, tuberculosis, and nephritis, in a large proportion of carcinoma cases and in a rather smaller proportion of sarcomas the cachexia is unaccompanied by hyperleukocytosis unless there is distinct local inflammation, necrosis, or hemorrhage. He suggests that the existence of a marked hyperleukocytosis in the course of a cachexia should lead to a search for one of these complications. Kast has described a remarkable instance of universal carcinomatosis with bone-marrow involvement in which the total number of leukocytes rose to 120,000, with 94.49 per cent. of neutrophiles, although septic complications did not occur.

4. *Antemortem Hyperleukocytosis.*—An agonal hyperleukocytosis in the old sense of the term is now no longer accepted. If during the agone hyperleukocytosis exists it is dependent directly upon the character of the morbid process, and not upon the agonal condition.

5. *Hyperleukocytosis referable to Drugs.*—Hyperleukocytosis referable to an increase of the polynuclear neutrophiles has been observed in cases of poisoning with potassium chlorate, arsenious hydride, illuminating gas, and coal-tar derivatives, such as antifebrin, phen-

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[1] Jour. Amer. Med. Assoc., July 19, 1902, p. 131.

[2] Diefendorf, Amer. Jour. Med Sci., December, 1903.

acetin, etc. It follows the administration of atropine, quinine, the salicylates, thyroid extract, tuberculin, and the infusion of salt solution. It is noted after prolonged anesthesia with chloroform and ether, when an increase of 5000 to 10,000 cells is quite common. This increase occurs after from six to forty-eight hours following the operation, and persists for only a few hours. A postoperative increase of 10,000 or more beyond the normal value of the individual, and sustained for more than a few hours, should be looked upon with suspicion[1] unless the case was a septic one from the start, when it may persist for several days.

6. *Hyperleukocytosis of Thermic Fever.*—In thermic fever a high leukocyte count is apparently the rule, but there is considerable irregularity in the time and duration of the rise. Lewis and Packard[2] report that in some of their cases a leukocytosis of from 12,000 to 13,000 was noted on admission. In most of the cases in which there was a primary rise this was followed by a fall and then a second increase in their number.

Polynuclear Neutrophilic Hypoleukocytosis (Leukopenia).—A diminution in the total number of the leukocytes is observed in only a comparatively small number of diseases, and is practically always referable to a decrease in the number of the polynuclear neutrophiles. It is notably observed in typhoid fever, measles, influenza, in certain anemic conditions, etc.

In *typhoid fever*[3] hypoleukocytosis is so constantly seen that we can formulate the general rule that *whenever an increase in the number of the leukocytes is observed in a case of suspected typhoid fever it is more than probable that some complication exists or that the diagnosis is wrong.* Exceptions to this rule are rare. In the very earliest days of the disease, possibly owing to a concentration of the blood, the result of starvation and diarrhea, higher counts are sometimes observed, but as the disease progresses the number soon diminishes, and in the later stages of the disease is practically always markedly below the normal. Not uncommonly they are less than 2000, and in some instances the number may indeed fall below 1000. The qualitative changes are especially important and fairly characteristic of the different stages of the disease. At first, while the temperature is steadily rising there is a neutrophilic hyperleukocytosis of moderate degree; this is associated with a moderate decrease of the lymphocytes, while the eosinophiles disappear. Then the neutrophiles diminish and the period of the hypoleukocytosis properly speaking commences. During this stage, viz., the stage of continued fever, the neutrophiles usually number from 3000 to 4000, as compared with 5000 to 6000 during the second half of the first week. The lymphocytes are now also diminished, but tend to rise toward the end of this period; the eosinophiles are absent. During the third stage (remission) the neutrophiles decrease still further—1500 to 2500—while the lymphocytes increase and a few eosinophiles appear. In the fourth stage (defervescence) the neutrophiles reach their minimum, 900 in severe cases, while the lymphocytes are relatively much increased and the eosinophiles gradually return to normal. The reascent of the neutrophiles then occurs very slowly, while coincidently there is a lymphocytosis which is especially marked in children and continues far into convalescence. Normal values are sometimes not reached until after a couple of months.[1]

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[1] Da Costa and Kalteyer, Amer. Med., 1901, p. 306.

[2] Trans. Assoc. Amer. Phys., 1902, p. 409.

[3] Nägeli, Deutsch. Archiv, lxvii, parts iii and iv. Kölner, ibid., lx, p. 221. Thayer, Johns Hopkins Hosp Bull., 1901, vol. iii, p. 500; and Studies in Typhoid Fever, Johns Hopkins Press, 1901, p. 487.

In the event of a relapse occurring during an afebrile period there is a distinct neutrophilic hyperleukocytosis, the actual number depending upon the preceding counts, to which from 3500 to 5000 neutrophiles are added; at the same time the eosinophiles disappear. Should a relapse occur in the third stage of the disease, then the eosinophiles, which have just begun to reappear, disappear abruptly.

Favorable indications in cases of typhoid fever are an increase of the eosinophiles at the height of the disease; reappearance of the eosinophiles, indicating arrival at the third or fourth stage; an increase of the lymphocytes, which appears to begin only at a time when the severest part of the intoxication is over; not too great a decrease of the neutrophiles in the absence of complications. Unfavorable indications are: a marked decrease of all leukocytes, and especially of the lymphocytes; absence of hyperleukocytosis and a further decrease of the neutrophiles in the event of complications, which *per se* would call forth a hyperleukocytosis.

In the event of complications the total number of the leukocytes frequently does not exceed the upper limit of the normal; but in such cases a differential count will reveal a relative increase of the neutrophiles.

In cases of perforation there is frequently an increase in the total number of the leukocytes, which may, however, be quite transitory and escape observation unless an early examination is made and previous counts are available; for later, when peritonitis is general, the leukocytes are usually found diminished. In some instances there is no increase at the onset.

In one of Cabot's cases the count before operation was 8300, and immediately afterward 24,000. Finney reports a case with 6500 before and 10,600 after. In one of Cushing's cases there was an early recognized hyperleukocytosis which appeared before any sign of general peritonitis had developed; 8400 before and 16,000 after. In this patient it was interesting to note that following the operation the leukocytes fell to 4000; but immediately following the development of obstruction, due to kinking of the bowel, the leukocytes increased to 13,000 and later to 20,000, to fall again following the removal of the obstruction. In a second case operated by Cushing there was a persisting hyperleukocytosis, associated with abdominal pain and tenderness, at one time reaching 15,200. Upon the development of general peritonitis the count showed only 4300. Cabot remarks, "Steadily increasing leukocytosis is always a bad sign, and should never be disregarded, even when other bad symptoms are absent," to which Cushing adds, "A decreasing leukocytosis may be a much worse sign" (Finney[1]).

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[1] In my experience the increase of the mononuclear elements affects not only the small mononuclears, but also the large mononuclears.

In *paratyphoid fever* the blood condition is essentially the same as in typhoid fever, viz., hypoleukocytosis early in the disease, disappearance of the eosinophiles, and later a marked increase of the lymphocytes which continues well into convalescence.

Measles[2] is the second notable exception to the general rule that the acute infections are associated with a polynuclear neutrophilic hyperleukocytosis. But it is interesting to note that here also the hypoleukocytosis is preceded by a preëruptive *hyperleukocytosis*, which commences at the beginning of the period of incubation, then increases rapidly and reaches its maximum about the sixth day before the appearance of the eruption. After this it diminishes, and at the appearance of the exanthem and during its course the occurrence of an increased number of leukocytes indicates some complication. The hypoleukocytosis affects the polynuclear neutrophiles both absolutely and relatively, while the lymphocytes are relatively at least increased. The eosinophiles disappear. The hypoleukocytosis generally reaches its maximum on the second day of the eruptive stage, when the number of leukocytes is reduced to about one-half. After this they increase again more or less rapidly and reach the normal one to five days after the disappearance of the rash, unless some complication should supervene. Numerous eosinophiles then appear together with an absolute and relative increase of the polynuclear neutrophiles.[3] Manicatide and Galasescu[4] do not share the generally accepted idea of the decrease of the leukocytes during the eruptive stage of measles. They maintain that a mild increase is usual during this time, which then disappears with desquamation.

Urticaria, syphilitic roseola, scarlatina, and the exanthem which may follow antitoxin treatment are not associated with hypoleukocytosis.

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[1] Surgical Treatment of Perforating Typhoid Ulcer. Studies in Typhoid Fever. Johns Hopkins Press, 1901, p. 170.

[2] Reckzeh, Zeit. f. klin. Med., vol. xlv, p. 107 (full literature).

[3] Renaud, Thèse de Lausanne, 1900.

[4] Folia hæmatol, vol. i, p. 110.

In uncomplicated cases of *tuberculosis* there is usually no increase of the leukocytes; when it does occur it is generally referable to suppurating cavities, recent hemorrhages, and resulting anemia, or to advancing pneumonia. The increase which occurs under such conditions is moderate and does not often exceed 15,000 cells. Ewing states that he has seen both lungs consolidated and riddled with small cavities in a case lasting but five weeks, and yet the leukocytes were never found above 12,000. He suggests that the absence of leukocytosis in such cases of acute phthisis which resemble pneumonia may often be of value in diagnosis. Unfortunately there is no large series of examinations available from which to decide the relative value of the morphological examinations of the blood in the differential diagnosis between acute miliary tuberculosis and typhoid fever. According to Cabot and Warthin, a subnormal number of leukocytes may also be observed in acute miliary tuberculosis, while Kölner[1] thinks the leukocyte count important in distinguishing between the two diseases.

In Malta fever the number of the leukocytes is usually not increased; some authors report that there is hypoleukocytosis as in typhoid fever.[2]

In uncomplicated cases of cachexial fever (Kala-azar) the leukocytes according to Rogers are markedly decreased. In India a number smaller than 2000 is regarded as almost diagnostic of the disease, but this may occur also in the true malarial cachexia. Rogers regards a reduction of the ratio of the whites to the reds to below 1 to 1500 as quite characteristic of cachexial as compared with other Indian fevers. The more marked the hypoleukocytosis and the reduction of the neutrophiles the worse the outlook. The neutrophiles usually number from 40 to 50 per cent.[3]

Carpenter and Sutton report low values in dengue, viz., 4460 as average with 1886 as minimal.[4]

In uncomplicated cases of *influenza* the total number of leukocytes is commonly diminished; it may, however, be normal. When an increase beyond 15,000 occurs, some complication probably exists. During the course of the disease I have noted the existence of lymphocytosis, with low eosinophile values. During convalescence the neutrophiles may show maximal normal values.

Hypoleukocytosis is one of the most constant symptoms of *pernicious anemia,* during the active period of the disease. At times it may be extreme. Strauss and Rohnstein[5] cite two cases with 400 and 328 cells respectively. As a rule it is much more moderate, viz., 2000 to 3000 cells per cubic millimeter.

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[1] Loc. cit., p. 96.

[2] E. Axisa, Centralbl. f. inn. Med., 1905, No. 11.

[3] Brit. Med. Jour., April 1, 1905.

[4] Jour. Amer. Med. Assoc., January 21, 1905.

[5] Loc. cit.

In *splenic anemia* also hypoleukocytosis is a common feature at some period in its course and is at times most marked. Osler mentions a case of Vickery's in which only 650 to 700 leukocytes were counted per cubic millimeter, and one of Peabody's with 800 cells. The average count in the series collected by Osler was 3850.[1] Immediately after a profuse hemorrhage or in a terminal infection there may be a hyperleukocytosis.

In other types of severe anemia hypoleukocytosis is less constant.

While the hypoleukocytosis in the diseases mentioned is rarely extreme, most extraordinary instances of leukopenia are at times encountered. Ehrlich[2] cites the case of a well-built young man in whom brief epileptiform seizures occurred, and in one of which the patient died. The postmortem examination was entirely negative. During the three days preceding death two examinations of the blood were made. On the first not a single leukocyte could be demonstrated in ten blood films, and on the second day but one was found in the same number of specimens.

Of *drugs*, atropin, camphoric acid, tannic acid, picrotoxin, agaricin, menthol, sulphonal, and several other antihydrotics, cause a marked decrease of the leukocytes.[3]

Neutrophilic Karyomorphism.—I have pointed out before that the neutrophilic elements of the blood can be subdivided into five classes according to the number of nuclear divisions (p. 81), and that the percentage figures representing these classes are normally quite constant for one and the same individual. Arneth has shown that in disease marked deviations from these normal standards may occur, and that the qualitative changes may be most pronounced even though there be no quantitative changes in the total number of the leukocytes, and vice versa. He accordingly distinguishes between, *iso-, normo-, hyper-, and hypocytosis, and aniso-, normo-, hyper-, and hypocytosis,* the term *iso* and *aniso* having reference to a normal or abnormal nuclear picture, respectively. Arneth's results are very interesting and show conclusively that the absolute leukocyte count *per se* is relatively of little importance, and that a more detailed morphological study of the blood is necessary in order to derive all the information possible from the blood examination. I have myself insisted for years that of the two the differential count is more important and from my experience with Arneth's nuclear studies I am quite prepared to admit that his method will at times furnish information of value, even when the differential count shows but little abnormality.

When alterations in the nuclear picture do occur the change usually first affects the maturest forms, viz., group 5; then follow the others until in extreme cases the youngest forms largely remain. As anisohypocytosis, according to Arneth, represents the most serious condition so far as the leukocytic blood picture goes, as it indicates both an extensive destruction of leukocytes and a defective new formation. Less serious would be an anisonormocytosis, more favorable an anisohypercytosis, and most favorable an isohypercytosis.

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[1] Amer. Jour. Med. Sci., 1902, vol. cxxiv, p. 751.

[2] Die Anæmie, loc. cit.

[3] Bohland, Centralbl. f. inn. Med., 1899, No. 15.

Anisohypocytosis occurs in fatal cases of pneumonia, constantly in typhoid fever and measles, frequently in varicella and mumps, further in severe cases of septicemia, in septic diphtheria, miliary tuberculosis, in acute articular rheumatism, fulminating appendicitis, and variola in the initial and eruptive stages.

In malignant diseases, so long as no complications exist, no special influence upon the nuclear picture can be demonstrated. If complications or marked metastases occur corresponding changes occur.

Polynuclear Eosinophilic Hyperleukocytosis (Eosinophilia).[1]—A *physiological* increase of the eosinophiles beyond the maximum observed in adults is seen in young children. According to Zappert, the relative numbers may here vary between 0.67 and 11 per cent., and Müller and Rieder even speak of 21 per cent. Personally I am not prepared to admit that such high figures occur in normal children. I rather imagine that some of these instances were cases of worm infection. In older children normal adult values prevail, and it is then legitimate to consider an increase beyond these figures as abnormal.

It is stated by some that there is a physiological increase of the eosinophiles during the menstrual period and following coitus. This is inconstant and rarely marked.

Eosinophilia is thus essentially a pathological phenomenon. It occurs under the most diverse conditions, as in myeloid leukemia, in bronchial asthma, in various skin affections, the helminthiases, gonorrhea, osteomyelitis, following the injection of tuberculin, etc.

In *myeloid leukemia* an absolute increase in the number of eosinophiles is one of the most constant symptoms. Ehrlich indeed has taught that this increase occurs in all cases and must be demonstrable to warrant the diagnosis. In view of recent advances in our knowledge of the pathology of the disease, however, this idea can no longer be upheld, as it has been shown that all forms of leukemia are or at least may be of myelogenous origin.[2] Cases have been recorded in which the blood picture was essentially that of the orthodox lymphatic variety, but in which postmortem examination showed a total absence of involvement of the lymph glands, while the bone-marrow was extensively diseased. In these cases there was no increase in the total number of the eosinophiles. But it seems that even in those cases in which the blood picture is essentially that of a myelemia the usual increase in the number of the eosinophiles may be lacking. I have reported an instance in which these cells were not only not increased, but practically absent.[1] Such cases, however, are exceedingly rare, and it may still be regarded as the rule that in those cases of leukemia in which extensive myelocytosis exists the eosinophiles are absolutely if not relatively increased. With septic complications occurring in the course of the leukemias the eosinophiles are materially diminished, and in some cases they may be absent. Exceptions, however, occur, and Ehrlich cites a case in which the absolute number of eosinophiles was still between 1400 and 1500 per cubic millimeter, although the percentage had fallen from 3.5 to 0.43.

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[1] For a thorough review of the literature on eosinophilia see C. E. Simon, International Clinics, 15th series, vol. iv.

[2] Pappenheim, Zeit. f. klin. Med., vol. xlvii, p. 216

In *bronchial asthma* an increase of the eosinophiles is observed quite constantly about the time of the paroxysm, and may amount to from 10 to 53.6 per cent.[2] Its occurrence is of value in differential diagnosis, as renal, cardiac, and diabetic asthmas are not associated with eosinophilia. Between attacks the numbers may be normal or increased.

In *scarlatina*[3] an increased number of eosinophiles is quite constantly observed at some time in the course of the disease. As the result of an anlaysis of 167 cases Bowie finds that at the onset of the fever they are diminished. In simple favorable cases they then increase rapidly until the height of the disease is passed, when they diminish again, and finally reach the normal some time after the general hyperleukocytosis has disappeared, viz., when the poison has all been eliminated. The more severe the case the longer are the eosinophiles subnormal before they rise again; in fatal cases they never rise, but rapidly decrease to zero. Bowie thinks that the curve of the eosinophiles is of value from a prognostic standpoint. If they are normal or subnormal after the first day or two, the case will in all probability be a severe one. In Reckzeh's series the highest percentage was 12.5, and the largest total number 1350.

In *measles* an increase of the eosinophiles does not occur.

In many *skin diseases* eosinophilia may also occur, especially in the bullous dermatoses, viz., Dühring's dermatitis herpetiformis, pemphigus foliaceus, and pemphigus vegetans, where 12 to 22 per cent. represent average values. In other skin diseases the tendency toward hypereosinophilia is also fairly pronounced, the degree of increase depending very largely, though not exclusively, upon the extent of the local process, as also upon the severity of the case. Light cases may thus show values which are practically normal. The list of diseases in which an increased eosinophile count has been noted includes herpes zoster, prurigo, eczema (as high as 45 per cent.), psoriasis, lichen ruber planus, urticaria (up to 60 per cent.), dermatoses of toxic origin—lead, mercury, picric acid, benzin (up to 31.5 per cent.), sclerodermia, mykosis fungoides (37 per cent.), lupus and lepra (8 to 61 per cent.). Exceptions, however, also occur.

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[1] C. E. Simon, Amer. Jour. Med. Sci., June, 1903.

[2] Billings, N. Y. Med. Jour., vol. lxv, p. 691.

[3] Zappert, Zeit. f. klin., Med., 1893, p. 292. Reckzeh, ibid., vol. xlv (literature). Bowie, Jour. Path. u. Bact., 1902, vol. viii, p. 82.

Sabrazès and Mathis have described eosinophilia in connection with a disease termed Ki-Mo, occurring in Tonkin and Laos.[1]

Leredde and Poutrier have observed eosinophilia in association with a skin eruption following the ingestion of antipyrine.[2]

Of special interest is the increase of the eosinophiles in the *helminthiases.* This is particularly marked in *ankylostomiasis* (*uncinariasis, hookworm infection*), where 72 per cent. were counted in one case. As a general rule, however, it is not so extensive. The eosinophilia in hookworm infection is very constant and unless other manifest symptoms exist which would suggest a different origin, its occurrence should always lead to a careful examination of the stools for the eggs of the parasite. I have repeatedly made a probable diagnosis of uncinariasis in persons coming from the sand region of the South, on the basis of a blood hypereosinophilia, where no other clinical symptoms and no anemia existed, but where subsequent examination of the feces bore out the correctness of the diagnosis. The increase of the eosinophiles may be out of all proportion to the number of the eggs found. This method I should suggest especially when large bodies of men are to be examined. The fecal examination can then follow in those cases, where blood examination shows the existence of a definite anomaly.

It is noteworthy that the hypereosinophilia of uncinariasis may be absent when the individual is greatly reduced by anemia.

From a study of 100 cases of uncinariasis in Porto Rico, Ashford[3] draws the following conclusions:

1. Eosinophilia occurs at some period in all cases. It is most marked in early cases and in late cases where blood regeneration is still active; 20 to 50 per cent. is then not uncommon.
2. In chronic cases or in those who have been profoundly anemic for a long time the eosinophile count is more apt to be low than high.
3. When there is a fall of eosinophiles, accompanied by a lack of improvement in the physical signs, death is apt to follow. A slow rise in eosinophiles marks a long convalescence.

In *bilharziasis* eosinophilia is also well pronounced. In 22 pure cases, uncomplicated by uncinariasis, reported by Kautsky-Bey,[4] the minimum percentage was 5 and the maximum 53. In the majority of cases the values were between 10 and 20.

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[1] Gaz. hebd. de Bord., 1903, p. 182.

[2] Soc. de biol., 1903.

[3] Amer. Med., 1903.

[4] Zeit. f. klin. Med., 1904, vol. lii, p. 192

In the presence of oxyurides Bückler[1] found 16 per cent.; 19 per cent. were counted in association with ascarides, and Leichtenstern reports one case of Tænia mediocanellata with 34 per cent. It is to be noted, however, that eosinophilia is not a constant feature in infections with the common tæniæ, oxyuris, and ascaris, and that the number may not exceed minimum normal values. In cases of infection with the bothriocephalus eosinophilia does not occur (Schaumann). This has been the experience also of others, at least in those cases in which active and pronounced anemia existed. After removal of the worm the eosinophiles may, however, temporarily increase beyond the normal, as in one of Gilman Thompson's cases[2] (9 per cent.).

In a fatal infection with Balantidium coli Strong and Musgrave[3] observed a relative increase, and it appears that in amebic colitis also a moderate eosinophilia is not uncommon.[4]

As Brown[5] has shown, a remarkable increase of the eosinophiles occurs in *trichinosis* during the acute stage. In his first four cases with a total leukocyte count of 35,000, 13,000, 17,000, and 18,000 the percentage of eosinophiles was 68.2, 42.8, 49, and 48, respectively. Kerr noted even a higher percentage in one case, viz., 86.6. Similar results have been obtained by Thayer,[6] Cabot,[7] Gwyn,[8] Blumer-Neumann,[9] and others, and it can now be regarded as an established fact that the occurrence of eosinophilia is one of the most constant and diagnostically important symptoms of the disease. It is in a general way proportionate to the intensity of the infection; when this is profound, however, the eosinophiles may not be increased and may indeed be diminished (Da Costa, Opie, Howard, Drake, and Cutler[10]). The eosinophilia persists for a long time (in my own case for three months). A very interesting case of trichinosis is reported by McCrae,[11] in which the disease was complicated by typhoid fever; the eosinophilia was here nevertheless well marked.

In *filariasis* also eosinophilia may occur. As the result of his study of four cases of the disease in the Philippines, Calvert[12] concludes that in the early stages hyperleukocytosis with an increase of the eosinophiles may be looked for, but that the number of the leukocytes in general, as also of the eosinophiles, returns to normal as the disease progresses. In one of his cases the percentage increased to 22, but varied within twenty-four hours between this point and 8. In a case of long standing which I had occasion to examine I found but 2 per cent. of eosinophiles, with 36 per cent. of lymphocytes. Calvert, on the other hand, noted an increase of the lymphocytes. A relation between the number of embryos and the percentage of the different leukocytes does not appear to exist.

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[1] Münch. med. Woch., 1894, Nos 2 and 3.
[2] Med. News, April 8, 1905.
[3] Johns Hopkins Hosp. Bull., 1901.
[4] Amberg, "Amœbic Colitis in Children," Johns Hopkins Hosp. Bull., 1901.
[5] Jour. Exp. Med., vol. iii, p. 315; and Johns Hopkins Hosp. Bull., 1897.
[6] Phila. Med. Jour., vol. i, p. 654.
[7] Boston Med. and Surg. Jour., vol. cxxxvii, p. 676.
[8] Centralbl. f. Bakt., vol. xxv, p. 746.
[9] Amer. Jour. Med Sci., vol. cxix, p. 14.
[10] Trans. Assoc. Amer. Phys., 1902, p. 356.
[11] Amer. Jour. Med. Sci., 1902, vol. cxxiv, p. 56.
[12] Johns Hopkins Hosp. Bull., 1902, vol. xiii, p. 133.

In hydatid disease the number of observations so far recorded is as yet too small to indicate the frequency with which hypereosinophilia is observed. Seligman and Dudgeon[1] report a case of hydatid disease of the liver with 57 per cent. of eosinophiles. Bloch[2] mentions one with 14.7 per cent., notwithstanding the fact that the cyst was suppurating. Still more recently Sabrazès[3] described three cases, all with hypereosinophilia, and still others are recorded by Audibert,[4] Dargein and Tribondeau,[5] Achard and Clerc—one case with 40 per cent.,[6] Achard and Laubry,[7] Frederici[8] and Meyer. That not all cases, however, are associated with hypereosinophilia is indicated by the negative reports of Bloch, Gourrand,[9] Launois and Weil,[10] and Besançon and Weil.[11]

Dr. J. Ramsey, of Launceston, Tasmania, has kindly sent me his findings in 5 cases of hydatid disease. In 4 of these there was no suppuration, but eosinophilia (28.4 per cent.) only occurred in 1. The others presented normal values; in 1 suppurating case no eosinophiles were seen.

In Medina worm (Guinea worm) infection eosinophilia has also been noted.[12] It is said to be present always in connection with the fever due to the presence of the worm.

It is generally stated that in *malaria* the eosinophiles are present in increased numbers during the afebrile period, and rarely diminish below the minimum normal values even at the time of a paroxysm. Zappert[13] reports a case in which on the day following the last attack 20.34 per cent. (1486 absolute) were found. Krauss[14] on the other hand, in an analysis of 204 cases of malaria, in nearly all of which the organism could be demonstrated, found but 2 per cent. on an average and supernormal values only exceptionally. Fontaine, who has had extensive experience in the study of this question in Louisiana, tells me that in uncomplicated malaria eosinophilia is but rarely seen.

In *malignant disease* eosinophilia apparently occurs in only a relatively small percentage of cases, and when present is usually of moderate grade—*i. e.*, not exceeding 7 to 10 per cent. Occasionally, however, the increase is most remarkable. Reinbach cites a case of lymphosarcoma (malignant lymphoma) of the neck with metastases in the bone-marrow, in which 60,000 eosinophiles were counted.

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[1] Lancet, June 21, 1902.

[2] Deut. med. Woch., No. 29, 1903.

[3] Gaz. hebdom. des sci. méd. de Bordeaux, 1903.

[4] L'éosinophilie, Paris, 1903.

[5] Soc. de biol., 1901.

[6] Gaz. hebdom., 1902.

[7] Soc. de biol., 1901.

[8] Rivista crit. di clin. med., 1902.

[9] Cited by Meyer (50).

[10] Soc. méd. des hôp., 1902.

[11] Arch. gén. de méd., 1902.

[12] Remlinger, Soc. de biol., July 9, 1904.

[13] Zeit. f. klin. Med., vol., xxiii, p. 227.

[14] Jour. Amer. Med. Assoc., October 22, 1904.

In the differential diagnosis of carcinoma from pernicious anemia I have found that an increase of polynuclear neutrophiles associated with a normal or supernormal eosinophile count is very suggestive of cancer.

A *gonorrheal eosinophilia* has been noted by various observers. From an analysis of 45 cases which Owings studied in my laboratory it appears that with an extension of the inflammatory process to the posterior urethra the number of cases increases in which an increased percentage of eosinophiles is found in the blood, and in cases of prostatitis eosinophilia is the rule. During the first week of the disease the blood is apparently always normal. In the second and third weeks it is normal in only 33 per cent. of all cases, and after two months' duration an increased number is still observed in 40 per cent. The percentage of the eosinophiles usually does not exceed 12 per cent. At times, however, larger numbers are found; Bettmann cites a case of gonorrheal epididymitis with 25 per cent. Occasionally the eosinophilia is associated with a neutrophilic hyperleukocytosis; this is usually of moderate intensity, but may be quite marked when the urethritis is complicated by an epididymitis, an orchitis, or a cystitis.

More commonly the neutrophiles are diminished in uncomplicated cases while the lymphocytes and often also the large mononuclear leukocytes are increased.

It is important to note that in gonorrheal arthritis also the eosinophiles are increased.

In association with chronic *tumors of the spleen* and after extirpation of the organ eosinophilia has been repeatedly observed. After extirpation eosinophilia is not immediately observed, however, but develops only after many months and is of moderate grade.

Granasso[1] claims that in *surgical tuberculosis* of children the eosinophiles are always increased, the number being largest in the lighter and in the convalescent cases. In the event of a complicating febrile disease or in connection with pyogenic infections a drop, of course, takes place. In a suppurating glandular case I recently counted 12 per cent.

As I have pointed out, the eosinophilic leukocytes are relatively diminished and may disappear altogether in the great majority of the acute infectious diseases, with the exception of scarlatina, while hyperleukocytosis referable to the polynuclear neutrophilic cells exists. In the postfebrile period, however, the upper limit of the normal and even a well-marked eosinophilia are often observed. Türk[1] found an *epicritic eosinophilia* of 5.67 per cent. (430 absolute) in a case of pneumonia, and after an attack of acute articular rheumatism 9.37 per cent. (970 absolute). I have seen an eosinophilia of 10.5 per cent after pneumonia.

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[1] Osped. Maria Vittorio, Torino.

An *eosinophilia referable to drugs* has been described, but has attracted little attention. Neusser mentions that pilocarpine will produce eosinophilia, as also iron, nuclein, and its derivatives, but gives no details. v. Noorden reports the occurrence of eosinophilia following the internal use of camphor (in two chlorotics). Similar observations have been made in animals after poisoning with carbon dioxide.

Following the injection of tuberculin an increase of the eosinophiles has been observed in those cases in which a febrile reaction had occurred. In one case reported by Grawitz the eosinophilia reached its highest point, viz., 41,000, three weeks after the injections had been stopped.

Polynuclear Eosinophilic Hypoleukocytosis (Hypo-eosinophilia).—A diminution in the number of the eosinophiles is notably observed in the acute infectious diseases which are associated with a neutrophilic hyperleukocytosis. The only exception to this rule apparently is scarlatina, but here also their number is diminished at the onset of the fever, and, as I have stated, in fatal cases they rapidly disappear. Aside from the infections which lead to an increase of the polynuclear neutrophiles, hypo-eosinophilia also occurs in those forms which, like measles and typhoid fever, are associated with a decrease of the leukocytes. We may accordingly formulate the general rule that a diminution in the number of the eosinophiles will be observed at some period in the course of the various acute infectious diseases, no matter whether they are associated with a general polynuclear hyperleukocytosis or not. The extent to which this may go is variable; in the milder infections the values may be but little, if any, below the minimum normal, but in the severer and more protracted cases not a single eosinophile may be met with in a differential count of a thousand. Whether or not cases occur in which they are wholly absent I am not prepared to say. I have pointed out before that I designate a neutrophilic increase associated with an eosinophilic decrease as the *septic factor*, and regard its demonstration as one of the most valuable symptoms in the diagnosis of pyogenic infections. In active appendicitis it is of constant occurrence and will serve to differentiate the condition from non-infectious abdominal affections.

Aside from the acute infectious diseases it is uncommon to meet with a material diminution of the eosinophiles. It has been observed after severe muscular exercise and after castration, and it is commonly noted in lymphatic leukemia with high lymphocyte counts. Da Costa[1] states that he has found a decrease or even an absence of eosinophiles in the majority of cases of chlorosis and pernicious anemia. This decrease in pernicious anemia has also been observed by others,[2] and is apparently the rule during the active stage of the disease; in the interval, however, normal and even supernormal values may be obtained. It is essentially seen in the kryptogenetic type, while in parasitic pernicious anemia the eosinophiles may be increased. In these cases a decrease may, however, also occur when the infection is very severe.

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[1] Klinische Blutuntersuchungen, Wien, 1898.

I have reported a remarkable case of atypical myeloid leukemia in which eosinophiles were practically absent.[3]

Lymphocytosis.—According to Ehrlich's conception of lymphocytosis as a *passive* hyperleukocytosis, an increased number of lymphocytes will be found in the blood in conditions which are associated with a hyperplasia of lymphadenoid tissue, the lymphocytes being mechanically washed into the blood current. But, as I have pointed out, there is evidence to show that the lymphocytes also may follow the laws of chemotaxis, and that an active lymphocytosis may possibly occur which is analogous to the hyperleukocytoses referable to the polynuclear granular elements.[4]

Under physiological conditions an increased number of lymphocytes is notably observed in early childhood. Following the temporary increase of the polynuclear neutrophiles which occurs during the first twenty-four hours, the lymphocytes rapidly increase in number, so that by the twelfth day they represent 45 per cent. of all leukocytes (Carstanjen). Gundobin gives 59 per cent. as an average value for sucklings as compared with 34.6 per cent. of polynuclear neutrophiles. In adult life a physiological increase of the lymphocytes is notably seen in connection with the increase of the polynuclear neutrophiles which occurs during the process of digestion.

Under pathological conditions lymphocytosis is more common in children than in adults, and it is noteworthy that in anemic and poorly developed children the normal ratio of lymphocytes to the polynuclear neutrophiles is reached only late. As a general rule the increase of the lymphocytes is not excessive and does not raise the total leukocyte count much above 30,000 to 40,000. Lymphocytosis of this order is notably seen in rickets, in whooping-cough, measles, congenital syphilis, in various subacute intestinal disorders of childhood, at times in bronchopneumonia, etc.

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[1] Clinical Hematology, Blakiston, Phila., 1901.

[2] Strauss u. Rohnstein, loc. cit., p. 31.

[3] Simon, Amer. Jour. Med. Sci., June, 1903.

[4] Jolly, "Sur les mouvements amœboides des globules blancs," etc., Comptes-rend. de la Soc. d. biol., 1898, vol. x, série v; and Wolff, "Giebt es eine aktive Lymphocytose," Deutsch. Aerzte-Zeit., 1901, No. 18.

In *whooping-cough* during the convulsive stage the total number of leukocytes may be increased to four times the normal; the average in De Amicis and Pacchioni's series[1] was 17,943. According to these observers, the hyperleukocytosis is demonstrable on the first day of the disease; it reaches its highest point in the convulsive stage and persists some time after cessation of the typical symptoms. Wanstall[2] in his series of 16 cases finds no evidence of a marked general hyperleukocytosis, and reports that in some the leukocytes were actually decreased. He could demonstrate a well-marked lymphocytosis during the catarrhal stage, however, in almost every case, which varied between 40 and 60 per cent. Wanstall concludes that an increased percentage of lymphocytes, at least equalling if not exceeding that of the polynuclear neutrophiles, is a valuable aid in the diagnosis of whooping-cough before the characteristic symptoms of the disease have appeared. Exceptions, however, occur, in which the lymphocytosis does not reach the usual high figures.

In *rickets* a well-marked lymphocytosis is the rule, which is both relative and absolute; the same holds good for *congenital syphilis* and for the secondary stage of the acquired disease.

In *bronchopneumonia* there is at times a well-marked lymphocytosis instead of a polynuclear hyperleukocytosis. Cabot cites an instance with a total leukocyte count of 94,600 and 66 per cent. of lymphocytes.

In *measles* there is at first an increase of the polynuclear neutrophilic elements; later the lymphocytes increase in inverse proportion to the neutrophiles, the total number being largely dependent upon the degree of glandular involvement.

In *typhoid fever* a relative lymphocytosis begins about the end of the first week and reaches its highest point in the stage of defervescence. Ewing states that he has found a uniform relation in this disease between the lymphocytosis in the blood and the grade of lymphatic hyperplasia found at autopsy. He records an instance in which the examination of the blood led to a strong suspicion of lymphatic leukemia, and in which at autopsy the mesenteric glands were of unusually large size, and the edges of the partly necrotic intestinal ulcers rose 1.5 cm. above the mucosa.

In smallpox there is a general tendency to an increase of the mononuclear elements. The same is seen in varicella.

In tuberculosis, when well advanced, the lymphocytes are usually diminished, and the more so the more prominently the patients have become septic. Early in the disease and in convalescent cases there is often a distinct tendency to lymphocytosis. In this respect my observations agree very well with those of Holmes.[3] In a series of 202 cases he found, associated with a lymphocyte count of 10 or lower, only 9 cases which could be viewed as recoveries or convalescents; with 10 to 20 per cent., 14 cases, and with more than 20 per cent., 39 cases.

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[1] Clin. Med. Ital., 1899, No. 1.

[2] Amer. Med., 1902.

[3] Jour. Amer. Med. Assoc., Jan. 28, 1905.

In uncomplicated *influenza* lymphocytosis is the rule during the active period of the disease, while in convalescence the neutrophiles may show maximum normal values.

In epilepsy there may be distinct hyperleukocytosis, referable to an increase of the small mononuclear elements. Boston and Pearce found the neutrophiles down to 29 per cent.

In pellagra mononucleosis apparently occurs with characteristic regularity, which may be of service in the diagnosis of the disease from other erythemas.[1]

In leprosy both lymphocytes and large mononuclear elements, but especially the former, are increased.

A relative as well as absolute lymphocytosis occurs in the helminthiases in which the eosinophiles are markedly increased. It is especially pronounced in trichinosis.

In general paresis, during the first stage, there is a tendency to hyperleukocytosis of the neutrophiles (70 to 80 per cent.); but in the third stage the latter fall as low as 40 per cent. while the lymphocytes are increased.[2]

A well-marked lymphocytosis is seen in Kala-azar.

According to Sahli a decrease of the total leukocytes, associated with a relative increase of the lymphocytes, may be observed in hemophilia.

In uncomplicated cases of *pseudoleukemia* an absolute increase of the leukocytes does not occur; but there is usually a relative increase of the lymphocytes of such extent that the normal ratio to the polynuclears 1 to 3 rises to 2 to 3 to 1. This relative lymphocytosis Ehrlich and Pinkus regard as characteristic of true pseudoleukemia, in the differential diagnosis from sarcomatosis and other lymphomatous growths.[3]

Grawitz,[4] on the other hand, maintains that from the leukocyte count no diagnostic conclusions can be drawn, and cites cases in which the ratio was either normal or in which the lymphocytes were actually diminished.

When the pseudoleukemic process involves the bone-marrow the blood findings may be very variable. As a result of stimulation of the myeloid tissue myelocytosis may occur; in other cases the blood picture closely resembles that of lymphatic leukemia (leukanemia, pseudopernicious anemia). In all such cases anemia is at the same time a prominent symptom owing to the replacement of the erythroblastic by lymphadenoid tissue.

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[1] Grigorescu and Galasescu. Spitalul, 1903.

[2] Bruce, Scott. Med. and Surg. Jour., June, 1903.

[3] Pinkus, Die Leukæmie, Nothnagel's Encykl.

[4] Klinische Pathol d. Blutes, 2d ed.

The highest grade of lymphocytosis is met with in the so-called *lymphatic form of leukemia.* As in the myeloid variety, the total number of leukocytes is here also very much increased, though not to the same extent. The highest count in Cabot's series was 220,000 and the lowest 40,000, so that we may regard 130,000 as an average. The lymphocytes usually number more than 90 per cent. In the chronic cases the small lymphocyte prevails, while in the acute cases the large lymphocyte controls the blood picture. When septic complications develop, the total number of the leukocytes, as in the myeloid form of leukemia, likewise undergoes a considerable diminution, but the lymphocytes still remain relatively increased. In one of Cabot's cases, in which as the result of septicemia the total number of leukocytes fell to 471 per cubic millimeter, the percentage of lymphocytes still was 94.7.

In the majority of cases of chloroma there is a moderate leukocytosis with lymphocytosis of the small or large cell variety, but in others myelocytes enter more or less prominently into the blood picture.

An *experimental lymphocytosis* has been observed following the injection of tuberculin and of extract of carcinomatous tissue (Grawitz). Waldstein claims to have produced a marked increase of the lymphocytes by hypodermic injections of pilocarpine, but, according to Ewing, this increase is only relative and brought about by a diminution of the polynuclear cells. Wilkinson speaks of a lymphocytosis following injections of quinine hydrochlorate and Perry has noted the same after the administration of thyroid extract.[1]

Lymphopenia.—Lymphopenia is notably observed in the acute infections which are associated with an increase of the polynuclear neutrophiles, and is almost always relative. The condition *per se* has received but little attention, and is relatively unimportant from the clinical standpoint.

Variations in the Number of Large Mononuclear Leukocytes.—Variations in the number of the large mononuclear leukocytes are as a rule not sufficiently marked to cause either a distinct increase or decrease of the total number of the leukocytes. One notable exception to this rule, however, exists in the cases of the acute type of lymphatic leukemia, in which the predominant cell is the large lymphocyte, viz., the juvenile form of the common large mononuclear leukocyte, in the sense of Pappenheim. At the same time it must be noted that some cases of chronic lymphatic leukemia also occur in which the large mononuclear leukocyte and Ehrlich's transition form represent a large percentage of the leukocytes. These relations, however, are not constant. A decrease is notably seen in myelocytic leukemia.

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[1] Cited by Da Costa.

In the so-called pseudoleukemia infantum of v. Jaksch a marked increase of the mononuclear elements is observed in a certain percentage of cases, but in the larger number the general increase of the leukocytes is referable to an increase of the polynuclear cells.

A relative as well as an absolute increase of moderate grade is observed in many of the diseases in which the lymphocytes are increased, as in rickets, syphilis, measles, scarlatina, smallpox, and according to my experience also in typhoid fever, etc. I have observed a marked increase in a case of Addison's disease a few days before death, and found notable numbers in debilitated individuals, in association with sloughing epithelioma, etc.

In a fatal case of epidemic cerebrospinal meningitis with a high leukocytosis and polynucleosis which I recently saw there was both a relative and absolute increase of the mononuclear leukocytes. Some of these as well as some of the neutrophiles contained meningococci.

In mycosis fungoides an increase of the large mononuclear elements has been noted by Hodara[1] and Pappenheim.[2]

A distinct increase is further observed in chronic malaria. In this connection Krauss[3] remarks that it is not so much the absolute increase of these cells which is diagnostic of malarial infection as the relative increase over the small mononuclears. In cases of malarial infection without much fever and without quinine the polynuclears are at the same time markedly diminished, while during the rise of a malarial fever, or as a result of quinine therapy, the polynuclear neutrophiles may reach 80 per cent.; but even then the large mononuclears exceed the small mononuclears.

In Kala-azar, as in malaria, there is a distinct tendency to an increase of the large mononuclears. In a series of 10 cases reported by Donovan, the average was 21.58 with variations from 6 to 48 per cent.

Variations in the Number of the Mast-cells.—A small number of mast-cells is found in the blood under normal conditions. The presence of more than 1.5 per cent. is probably always pathological. A remarkable increase is noted in the myeloid type of leukemia and is one of the most constant features of the disease; more constant, in fact, than the increase of the eosinophiles. The percentage is not necessarily above normal, but not infrequently values of from 5 to 10 per cent. are found. It is noteworthy that this increase of the mast-cells may be demonstrable at a time when the disease is apparently quiescent. In one instance of this kind the total number of the leukocytes had been 350,000; three months later I counted but 2080, of which 10.9 per cent. were mast-cells, and later they rose to 15 per cent.

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[1] Monatsheft f. prakt. Dermat., 1904.

[2] Folia hæmat., vol. i, p. 487

[3] Jour. Amer. Med. Assoc., October 22, 1904.

The only other condition in which I have found such high values occurred in a patient, following fracture of the ankle and consequent cellulitis. In this case they rose to 17 per cent. and the blood in general presented a typical leukemic picture. A few days later normal values were found.

A more moderate increase is noted in many other diseases. Generally speaking, my experience has been that they are more numerous in conditions in which the eosinophiles also are increased, and are generally diminished when the eosinophiles are below normal. This rule, however, is not absolute. I have found values above the normal in various skin diseases, in gonorrhea, in certain cases of malignant disease, associated with eosinophilia. In one case of renal carcinoma a few weeks after the removal of the growth I counted more than 2 per cent. of mast-cells, with but 1.9 per cent. of eosinophiles.

Canon reports an increase of mast-cells in chlorosis; Sherrington, in cases of Asiatic cholera, dying in the reactive stage; Taylor, in two cases of septic bone disease; Da Costa states that an increase has also been observed in some cases of splenic anemia.

I have found the number diminished or entire absence of mast-cells in some cases of malignant endocarditis, appendicitis, empyema, influenza, tonsillitis, intestinal obstruction, lumbar abscess, periproctitic abscess, pernicious anemia, hematoma of the abdominal walls, diabetes, carcinoma of the cervix (septic), "black" jaundice, pneumonia (unresolved). In malaria the number is usually normal.

Myelocytosis.—At birth and during the first weeks of life it is usual to meet with a small percentage of *neutrophilic myelocytes* in the circulating blood under perfectly normal conditions, while in adults their presence is always abnormal. In children the tendency to myelocytosis is always more pronounced than in adults. Zelänski and Cybulski,[1] who have studied this question more particularly, have found myelocytes in a great many diseases. In the pseudoleukemia of v. Jaksch the number varied between 1.5 and 17.4 per cent., in congenital debility from 3.5 to 12.5 per cent. In congenital syphilis they found myelocytes quite commonly, the number—usually moderate—depending upon the intensity and duration of the morbid process. They disappear upon institution of mercurial treatment. In rickets the number of myelocytes is dependent upon the intensity of the morbid process. In the lighter cases they are scanty or absent. Scrophulosis is not associated with myelocytosis. Tuberculosis and catarrhal processes involving the digestive apparatus, of long duration, cause the appearance of myelocytes in fairly large numbers. Very curiously acute dyspeptic processes were also found associated with myelocytosis in very young children. In a case of congenital atresia of the anus they found 20 per cent. of myelocytes.

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[1] Jahrb. f. Kinderheilk., 1904, vol. lx, p. 884

Türk has shown that they are quite common in the acute infectious diseases of childhood, and in diphtheria Engel ascertained that they are especially numerous in the severe cases (3.6 to 16.4 per cent.). In mild infections they are not usually seen, and when present they are found in only very small numbers. In pneumonia they are absent or very few in number at the beginning of the disease, while at the time of the crisis or immediately thereafter they become more numerous and in some cases represent 12 per cent. of all neutrophilic cells; such high percentages, however, are rather uncommon and are more apt to be encountered in children than in adults. In acute septic conditions a small number of myelocytes may also be observed; larger numbers are found in the more chronic cases, which are associated with marked anemia. In a case of lumbar abscess which had been discharging for six months I found 7.8 per cent.

In a case of "black" jaundice I found 2.2 per cent. Neusser has noted their presence in asphyxia and acute mania; Ewing states that they have been found in considerable numbers in rickets, osteomyelitis, and osteomalacia. Da Costa speaks of their occurrence in poisoning by carbon monoxide, in hepatic cirrhosis, acute gout, malignant endocarditis, and exophthalmic goitre.

The occurrence of myelocytes under these conditions is to be regarded merely as a quantitatively or gradually increased polynucleosis of the corresponding granular cells, the result of an increased destruction of the adult cells and consequent increased production (anisohypercytosis). This is in contradistinction to the myelocytosis associated with myeloid leukemia where there is a primary increased formation referable to myeloid hyperplasia; this form is essentially a passive myelocytosis, while the former is active.

In anemic conditions of whatever origin it is common to meet with a moderate number of neutrophilic myelocytes. In pernicious anemia they are quite constant in the active stage of the disease; as a rule the values do not exceed 0.5 to 1 per cent., but at times they may reach 7 per cent.

Pappenheim makes a distinction between primary hemophthisic pernicious anemia of the Biermer type and the form referable to bothriocephalus infection, on the one hand, in which myelocytes in his experience do not occur, and primary myelophthisic splenomedullary anemia, myelomatosis, and myelogenous pseudoleukemia (tumor anemia, pseudopernicious anemia) on the other, in which they may be present.

According to Kurpjuweit[1] the occurrence of myelocytes in large numbers (4 to 17 per cent.) in connection with the symptom complex of a severe anemia is to be viewed as almost pathognomonic of malignant growth with bone-marrow metastases, even when a primary tumor cannot be found.

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[1] Deutsch. Arch., vol. lxxvii.

In the secondary anemia associated with syphilis and malignant disease, as also in the pseudoleukemia of v. Jaksch, variable figures are found (1.5 to 17 per cent.). In a young child in which a notable anemia had developed as the result of amebic dysentery, Amberg counted 9 per cent. In the estivo-autumnal type of malaria they are quite common.

The neutrophilic myelocytes which are met with under these various conditions are almost without exception of the small trachychromatic variety. The amblychromatic variety is practically only encountered in the myeloid type of leukemia, which is really the one disease in which large numbers of myelocytes of all kinds find their way into the blood. Upon their presence in numbers exceeding those found in all other diseases the diagnosis is largely dependent. The blood state is that of a true *myelemia.* The number of neutrophilic myelocytes in myeloid leukemia is often most remarkable, and a count of from 50,000 to 100,000 per cubic millimeter is by no means exceptional. The average percentage of 18 cases reported by Cabot was 37.7, corresponding to a total number of 162,000 leukocytes. Coincidently with the neutrophilic myelocytes eosinophilic myelocytes also appear in the blood and may constitute the majority of the eosinophilic cells seen in this type of the disease; their percentage, however, is rarely large. The total number of the polynuclear eosinophiles is at the same time increased, although the relative percentage may be normal or even slightly below normal. The polynuclear neutrophilic cells and the lymphocytes, while absolutely increased, are relatively much diminished. Of the latter, only 7.6 per cent. are found on an average, and of the former 49.2 per cent., as compared with 20 to 30 and 60 to 70 per cent., respectively, under normal conditions. The mast-cells, as I have pointed out, are invariably present in increased numbers in the myeloid type of the disease.

While the majority of the neutrophilic and eosinophilic cells present a normal habitus, it is common in myeloid leukemia to meet with dwarfed forms. Occasionally also leukocytes are observed which are undergoing mitosis. Of special interest is the fact that in certain chronic cases of the disease the neutrophilic cells apparently lose the power of forming neutrophilic material. Non-granular polynuclear cells and myelocytes then appear in the blood and may give rise to much confusion. In one case of this kind reported by Ehrlich the great majority of the mononuclear elements, which constituted 70 per cent. of the total number, were entirely free from neutrophilic granules.

The total number of the leukocytes in myeloid leukemia in the active stage of the disease is much increased. In Cabot's series of 30 cases the average was 438,000. If at the same time, as not infrequently occurs, there is a coincident anemia with marked diminution of the red cells the ratio between the whites and reds may fall to 1 to 2 or even 1 to 1; there are cases on record, indeed, in which the leukocytes outnumbered the red cells. Formerly much stress was laid upon this ratio in the diagnosis of the disease; leukemia was regarded as a hyperleukocytosis in which the ratio exceeded a definite proportion that was generally placed at 1 to 50. As a matter of fact, there is probably no other disease in which so great an increase of the leukocytes is observed, and even at the present day the diagnosis is usually justifiable when an increase of such proportions is noted. But, as I have pointed out, myeloid leukemia is essentially a myelemia and not a hyperleukocytosis. There are cases, moreover, exceptional to be sure, in which the increase of the leukocytes is not so extreme. I have observed one case in which the total number was only 2080 and the ratio of the whites to the reds 1 to 1015. The diagnosis of the disease should hence be based primarily upon qualitative changes in the morphology of the blood and only secondarily upon an increase of the leukocytes as a whole.

When septic complications supervene in the course of the disease the blood condition may undergo marked changes. Thus, in proportion to the degree of infection the myelemic picture gradually disappears and is replaced by that seen in simple septic conditions. The polynuclear neutrophiles may then increase to 90 per cent., and even more, while the eosinophiles diminish and may almost disappear.

X-ray treatment in a certain number of cases may cause a marked change in the blood picture. The total number may fall rapidly and there is a general tendency to normal conditions; a complete disappearance of myelocytes is, however, very rare. The mast-cells very curiously remain above normal.

In the purely lymphatic form of leukemia neutrophilic myelocytes are scanty; there are cases of mixed leukemia, however, in which at some stage of the disease the blood picture is essentially of the lymphatic type, while at another period there is a marked myelocytosis.[1]

In a case of compound fracture with consequent cellulitis I found a blood picture which was typical of myelocytic leukemia, with large numbers of myelocytes (15 per cent.). After a few days there was a return to normal. Hastings tells me that he has found myelocytes in 3 to 7 per cent. of cases of fracture.

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[1] For a detailed consideration of the blood changes in leukemia see especially: Pinkus, "Die Leukaemie," Nothnagel's Encycl. Ewing, Clinical Pathology of the Blood, Lea Brothers. Cabot, Clinical Exam. of the Blood, Wm. Wood & Co. Pappenheim, Zeit. f. klin. Med. Haematologische Streitfragen, 1903.

In pseudoleukemia myelocytes are essentially seen in cases where the pathological process has affected the bone-marrow (myeloid pseudoleukemia) and where, as a result of lymphadenoid substitution of the myeloid tissue, an irritative myelocytosis develops.

Eosinophilic myelocytes, aside from their occurrence in myeloid leukemia, are comparatively rare. They have been found in the pseudoleukemia of infants; Mendel[1] speaks of their occurrence in a case of myxedema; Türk[2] reports that they are occasionally seen in some of the infectious diseases, and Bignami claims to have seen them in pernicious malaria. In one case of posthemorrhagic anemia referable to a ruptured tubal pregnancy I found 1 per cent. of eosinophilic myelocytes, and in a case of myelogenous leukemia, in which the eosinophiles were absolutely much diminished, the only eosinophile that I could find in many slides was a myelocyte. In a case of trichinosis they were also occasionally seen at a time when the eosinophiles were much increased.

The Plaques.

In addition to the leukocytes and red corpuscles large numbers of small, roundish elements are encountered in the blood which measure about 3 μ in diameter and are free from coloring matter (Plate II, Fig. 1). They are frequently seen collected into groups resembling bunches of grapes. These are the blood plates or plaques of Bizzozero. According to Hayem, they represent red corpuscles in an early stage of development, and are themselves derived from leukocytes within the lymph channels. He terms them *hematoblasts.* This view is not shared by modern hemotologists. Lilienfeld, Hauser, Howell, and others regard the plaques as disintegration products of leukocytes, while still others look upon them as precipitated globulins derived in part from the morphological elements of the blood and in part originating directly in the plasma. More generally accepted is the view expressed by Engel, Bremer, Maximow, Pappenheim, and others, according to which the plaques are derived from the red cells by extrusion. They are originally contained in the interior of the cells as so-called nucleoids, and represent the remains of the original nucleus, which has lost its individuality as the result of chromatolysis. As a matter of fact, it is possible by suitable staining to demonstrate the plaques not only within the red cells, but also their extrusion from the cells, so that the erythroglobular origin of some of these formations at least can scarcely be doubted. Jost, moreover, has shown that in the blood of sheep and calf embryos they appear at a time when leukocytes are not as yet demonstrable. But, on the other hand, there is a possibility that what we generally designate as plaques does not represent a unity, and that some of the elements which resemble the true blood platelets may be of different origin. To a certain extent such ill-defined little bodies are without doubt derived from leukocytes by a process of plasmorhexis—*i. e.*, by the liberation of small bits of protoplasm. This may be observed under the microscope directly.

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[1] Berl. klin. Woch., 1896, No. 45.

[2] Klin. Untersuch. d. Blutes, etc., Wien u. Leipzig, 1898.

Deetjen has shown that the true plaques are capable of executing ameboid movements when the blood is placed on a slide which has been covered with a thin film of agar containing a certain amount of sodium chloride, sodium metaphosphate, and dipotassium phosphate. He also believes to have demonstrated a nucleus in the individual plaque, and concludes that the bodies in question do not represent artefacts or products of degeneration, but are true cellular elements.

According to Osler, the number of plaques varies normally between 200,000 and 500,000 per cubic millimeter. Brodie and Russell claim that this number is too small, and that with their improved method of counting an average of 635,300 is obtained. The normal ratio between the plaques and the red corpuscles would thus be 1 to 7.8, taking 5,000,000 as the average normal for the red cells. More recently Helber found variations between 192,000 and 264,000.

Under pathological conditions the plaques may be increased or diminished. In pernicious anemia their number is very low; Van Embden found 64,000 and 32,000 in two cases. At times they are apparently absent, but in some cases increased numbers have been observed.

According to Pappenheim the plaques are diminished in pernicious anemia owing to over-rapid maturation of the red cells. As a result the nuclei of the erythroblasts either do not become pyknotic and undergo chemical chromatolysis with consequent formation of oxyphilic, viz., azurophilic nucleoids, but are destroyed already in an early stage by karyorrhexis; or, if they do become pyknotic, they are expelled from the cells plasmolytically in the anisotonic (anemic blood serum). A nucleoid thus does not remain which could later escape as a plaque.

In leukemia the plaques are often greatly increased. A large increase is at times observed in posthemorrhagic anemia and in chlorosis, but the results are not constant. In the secondary anemias referable to carcinoma, sepsis, tuberculosis, etc., the findings are variable; sometimes an increase is observed, at others a decrease, and then again normal values; the results, moreover, are inconstant in one and the same case. In the acute infectious diseases their number is the smaller the more severe the course of the disease. In pneumonia they are often diminished during the fever, but increased after the crisis. Similar results have been obtained in typhoid fever, while in erysipelas they are found increased from the start. Enormous numbers of plaques may be seen in the course of trichinous infection. Schleip looks upon their appearance in large numbers as evidence of approaching convalescence. In my own case, however, they seemed to be most numerous at a time when the clinical symptoms were most active.

(For the enumeration of the plaques see p. 144.)

Literature.—Bizzozero, Virchow's Archiv, vol. xc. Hayem, Le sang, Paris, 1889. Howell, Jour. of Morph., 1891, vol. iv, p. 57. Maximow, Arch. f. Anat., 1899, vol. i, p. 33. Jost, Arch. f. mik. Anat., 1903, vol. lxi, p. 667. Determann, Deutsch. Arch. f. klin. Med., vol. lxi, p. 365. Deetjen, Virchow's Archiv, 1901, vol. clxiv, p. 239. Brodie and Russell, Jour. Physiol., 1897, Nos. 4 and 5. Heller, Deutsch. Arch., vol. lxxxi, Heft 3 u. 4.

The Dust Particles or Hemokonia of Müller.

These may be seen in any fresh specimen of blood mounted in the usual manner. They are small, generally round, sometimes dumb-bell-shaped, colorless, highly refractive granules, which manifest very active molecular movements. They occur in the plasma of the blood and are apparently not connected with the process of coagulation. Müller found them abnormally numerous in a case of Addison's disease, while they were diminished during starvation and in various cachectic conditions. Stokes and Wegefarth regard these granules as identical with the neutrophilic and eosinophilic granules of the leukocytes. They suppose that the bactericidal power of the leukocytes and of the serum of man and many animals is due to their presence. As a matter of fact, the origin of the hemokonia from the granular leukocytes can frequently be directly observed.

I have quite constantly found the hemokonia increased at the height of digestion, and have then repeatedly observed their extrusion from both neutrophilic and eosinophilic cells.

Literature.—H. F. Müller, "Ueber einen bisher nicht beachteten Formbestandtheil d. Blutes," Centralbl. f. allg. Path. u. path. Anat., 1896, p. 929 W. R. Stokes and A. Wegefarth, "The Presence in the Blood of Free Granules, etc., and their Possible Relation to Immunity," Johns Hopkins Hosp. Bull., 1897, p. 246. E. B. Sangree, "Leukocytic Granules," etc., Phila. Med. Jour., 1898, p. 472.

General Technique.

Slides and Cover-glasses.—To obtain satisfactory results, it is essential to have glassware of the best quality. The cover-glasses should not measure more than 0.08 to 0.10 mm. in thickness and must be cleansed with care. The same holds good for the slides, which should have a *level* surface; many of those furnished by dealers are unsatisfactory for work with immersion lenses.

Both covers and slides should be placed in concentrated sulphuric acid or in glacial acetic acid for several hours. They are thoroughly washed in running water and distilled water and then placed in alcohol and finally in ether, where they remain for several hours. During this process care must be had that they are well separated from each other. Subsequently they are kept in jars with absolute alcohol, and are dried just before use, or they may be dried at once with fine linen or Japanese lens paper and stored in dust-proof receptacles. When once cleansed, the cover-glasses should be handled only with forceps.

To cleanse slides that have been used, the covers must first be removed by immersion for several days in xylol or turpentine. They are then placed in hydrochloric acid to which about a teaspoonful of potassium chlorate has been added for every 30 c.c. The mixture is kept on the boiling water bath to the point of decolorization. The slides are next rinsed in hot water, heated for a half-hour in a thin mush of equal parts of washing soda, sawdust, and talcum, prepared with the aid of water and stirring frequently, then washed off with hot water acidified with hydrochloric acid, and finally with pure hot water, alcohol, and ether.

The Blood Mount.—We distinguish between wet mounts and dry mounts. Wet specimens can only be utilized successfully if the patient is near at hand to the laboratory, as in office work and in the hospital; where several hours must elapse before the preparation can be examined, it will usually be best to resort to the dry specimen. Wet preparations, however, are very convenient and yield a large amount of information without delay, and a rapid survey will indicate whether or not it will be necessary or advisable to resort to a more detailed examination. The grade of an anemia: the degree, character, and extent of a hyperleukocytosis; the presence of malarial organisms, can all be told from the wet preparation. With the dry and stained specimen, on the other hand, all these points are brought out more distinctly, and other information is further afforded which cannot be obtained from the wet specimen alone.

To prepare a blood specimen, the tip of a finger, or in children especially the lobe of the ear, is first cleansed with ether and then punctured with a suitable instrument, such as a fine lancet or a stout needle. The puncture should be sufficiently deep that the blood will flow from the wound without undue pressure.

To prepare a wet specimen, a clean cover-glass is taken up with a pair of forceps, with flat blades and a light spring, touched to the drop without coming in contact with the skin, and immediately transferred to a clean slide. If suitable glassware is used that is perfectly clean, the drop will immediately spread out between cover-glass and slide, and on examining with a low power, which should always precede examination with a high power, it will be noted that in the central portion of the specimen especially the red cells will be well separated from one another and will not have run into rouleaux. This will only occur if the glassware is imperfect, if it is not perfectly clean, or if the drop has been too large. To gauge the proper size of the drop requires a little practice. Along the margin of the specimen, where a certain amount of evaporation is going on, it is usual to find rouleaux and crenated red corpuscles, even though the rest of the specimen is perfect, and in the course of time postmortem changes will also become noticeable throughout the preparation. If the specimen is ringed with a little paraffin, however, a satisfactory examination is still possible after a number of hours, and even without being ringed such preparations can be kept for at least one hour.

To prepare dry specimens, which are subsequently to be stained, the blood is spread between cover-glasses or on slides.

Personally I have almost abandoned the use of cover-glasses, and much prefer slides for routine work. But little practice is required to obtain very satisfactory results, and it is possible to control the quality of the individual smears with a degree of precision which is but rarely attained even by the most experienced workers with cover-glasses. The spreads, moreover, are much larger; so that there will always be a sufficient number of leukocytes available even under normal conditions to permit a count of at least a thousand cells. At the same time it is possible to spread portions of the drop so thin that the individual cells are well separated the one from the other, while other portions can be made a little thicker. The slides are cleansed in the same thorough manner as in the case of the cover-glasses. A fair-sized drop of blood is then mounted near the end of

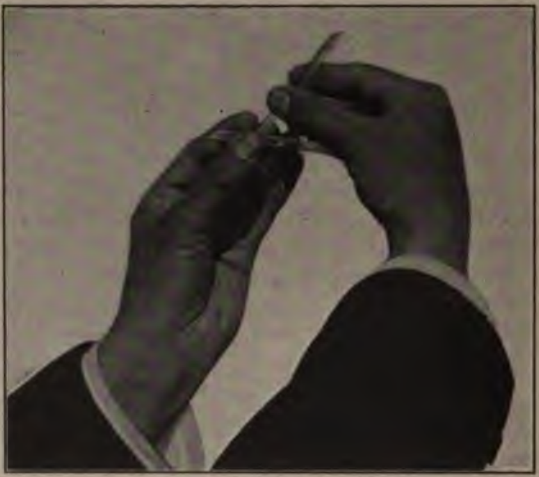


Fig. 17.—The preparation of blood smears on slides.

one slide and spread with an even sweep with the edge of a second slide; this should be done with a *light* hand, and holding the first slide in the left hand between the thumb and the second and third fingers. The second slide should also be held in this manner, but at an angle of 45 degrees to the first, as shown in the accompanying illustration (Fig. 17). Before commencing the sweeping movement I let the blood spread along the edge of the second slide by capillary attraction; then I move across, gradually raising the second slide to a vertical position. *Pressure must be carefully avoided.*

If covers are to be used, one cover-glass is locked in a pair of forceps such as those devised by Ehrlich and pictured in the accompanying illustration (Fig. 18). A second cover is taken up with a pair of forceps without a lock, but with flat blades and a light spring; this is held to the drop of blood just as it emerges from the puncture, and is then immediately laid upon the first cover. If the glasses are of satisfactory quality and clean, the blood will at once spread in a capillary layer; the top cover is then drawn from the lower cover by grasping the edge firmly with the fingers and making even traction in a plane parallel to the other. Here also a certain amount of experience is necessary in gauging the size of the drop in reference to the size of the covers. In no case should it be so large that the top cover *floats* upon the blood. If the drop is rather small, the two covers should overlap only to such an extent as to furnish a space which is just filled by the blood. If the drop is larger, they should overlap over a larger surface.

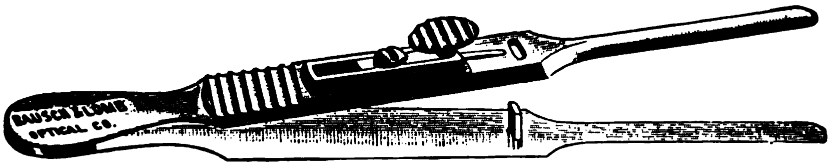


FIG. 18.—Ehrlich's cover-glass forceps.

After being allowed to dry in the air the specimens are placed between layers of filter paper and may then be stained at leisure. If several days must elapse before the examination, it is well to place them, wrapped in filter paper, in closed jars. Should it be desired to preserve the specimens for a long time—*i. e.*, for months or years—it is best to coat the films with a thin layer of paraffin, which later is dissolved by immersion in toluol. In this manner especially valuable and rare specimens may be kept almost indefinitely. Unless this precaution is taken, the staining qualities of all the morphological elements of the blood will undergo changes which render the specimens unfit for color analysis.

Fixation.—The selection of the method of fixation depends very largely upon the stain which is to be employed. If strongly alcoholic solutions are used no previous fixation is necessary, but with aqueous solutions fixation must precede the staining. To this end several methods may be employed. The best results are obtained by heat. For this purpose a copper plate may be used measuring about 10 cm. in width by 40 cm. in length and 3 to 5 mm. in thickness; this is heated by a Bunsen burner or a small coal-oil stove. After the plate has a fairly constant temperature, the desired degree is ascertained by a series of drops of water, toluol (boiling point, 110° to 112° C.), or xylol (137° to 140° C.), etc., noting the line at which ebullition occurs. If the distance of the plate from the flame and the size of the flame, etc., are constant, the apparatus requires practically no attention and serves its purpose very well. As a rule a brief fixation only is necessary—*i. e.*, exposure to a temperature of from 100° to 126° C. for one-half to two minutes, while in special cases Ehrlich recommends a more prolonged exposure or a higher temperature. Very good results are obtained for most purposes by heating the blood films to a temperature of 140° C. for thirty to forty-five seconds, as suggested by Rubinstein. This point is conveniently ascertained on the copper plate by noting the line at which the so-called Leidenfrost phenomenon begins to occur, viz., the point at which a drop of water assumes the spherical form and rolls about on the plate.

In the place of the copper plate an ordinary drying oven provided with a thermostat and thermometer or a so-called Victor Mayer Siedekessel may also be employed. The latter is a small copper kettle covered with a thin plate, which is perforated for the reception of the boiling tube. If a small quantity of toluol is boiled in this kettle for a few minutes, the copper plate will acquire a temperature of from 107° to 110° C., and retains this sufficiently long for ordinary purposes (Ehrlich).

Absolute alcohol or a mixture of equal parts of absolute alcohol and ether (Nikiforoff) have also been recommended as fixing agents for blood films, but are not very satisfactory for the study of the neutrophilic granulation. With Ehrlich's triacid stain especially it will frequently be noted that the granules are stained imperfectly or not at all. For the study of nuclear structures, however, both are quite satisfactory. In the case of absolute alcohol alone immersion of the blood films for a few minutes is sufficient; with alcohol and ether fixation for one-half to two hours is necessary.

Formalin is useful as a fixing agent and may be used in connection with practically all the common blood stains. A 1 per cent. alcoholic solution is employed. This is prepared by diluting one part of the commercial formalin, which is a 40 per cent. solution of formaldehyde gas, with nine times its volume of water, and one part of the resulting solution with nine times its volume of alcohol. Fixation is completed in one minute, and for practical purposes it is merely necessary to cover the blood films with a few drops of the solution, which is then drained off and replaced with the staining reagent directly. The method is not to be recommended, however, for routine purposes, as it interferes with various stains and often changes the normal chromatophilia. The same may be said of the use of concentrated solutions of bichloride of mercury, which also is useful for some purposes, but not for routine work.

If the dyes are used in alcoholic solutions, as with Jenner's stain, Hasting's, Wright's, or Giemsa's stain, no previous fixation is necessary.

The Aniline Dyes and the Principles of Staining.—The aniline dyes with which we have to deal in the clinical laboratory are all derivatives of hydrocarbons and all contain the benzol ring. Their staining properties are dependent upon the presence in the individual compounds of two distinct atomic complexes which are spoken of as *chromophoric* and *auxochromic* groups, respectively. The presence of the chromophoric group imparts chromogenic properties to the substance, the dye itself resulting on the further introduction of an auxochromic group. The auxochromic groups are salt-forming radicles and render the dye either basic or acid. Two markedly auxochromic radicles are known, viz., the strongly basic amino group —NH_2 and the feebly acid hydroxyl group —OH. Still other salt-forming radicles may enter into the composition of the dye, but it is noteworthy that these have but feebly developed auxochromic properties. Radicles of this order are notably the carboxyl group —COOH, the sulphoxyl group —SO_2OH, the nitro group —NO_2, and the nitroso group —NO (which two latter may also occur as chromophoric radicles). As the chromophoric radicle itself may have acid or basic tendencies it is manifest that the ultimate reaction of the individual compound will depend upon the inter-relation of the sum of its acid and basic radicles. Markedly acid dyes will result if both the chromophoric group and the salt-forming radicles are acid, while strongly basic dyes will be the outcome if both have basic tendencies. Between these two extremes various possibilities exist, the ultimate reaction depending upon the character of the chromophore, the presence of acid or basic salt-forming radicles, the simultaneous presence of both, their number, etc. We may accordingly divide the various dyes into the following classes:

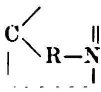
1. Basic amino dyes.
2. Acid nitroso dyes.
3. Acid sulpho- and nitro dyes, viz., amino- or oxysulphonic acids, aminoöxysulphonic acids, nitrophenols, nitroamins, nitroaminosulpho acids, nitroöxysulpho acids, nitroaminoöxysulpho acids.
4. Acid oxy- and oxycarbonic dyes.
5. Aminoöxy-, aminocarbonic, and aminoöxycarbonic dyes.
6. Aminosulphocarbonic-, oxysulphocarbonic-, aminoöxysulphocarbonic-, aminonitrocarbonic-, oxynitrocarbonic-, aminoöxynitrocarbonic-, and aminoöxysulphonitrocarbonic dyes.

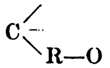
Of chromophoric groups, some twenty are known, and it is customary to classify the aniline dyes on the basis of these underlying radicles. A common characteristic of all chromophoric groups is the fact that they impart a quinone-like structure to the respective compounds. We find:

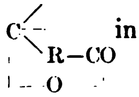
The $—NO_2$ group in the nitro dyes (picric acid, Martius yellow, naphtol-yellow S, aurantia).

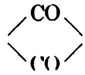
The —NO group in the nitroso dyes (Echtgrün, naphtol green).

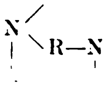
—N=N— in the azo dyes (aniline yellow, chrysoidin, vesuvin, Sudan G and III, alizarine yellow FS, Ponceau, Bordeaux, amaranth, coccinin, orange G, tropeolin, Biebrich scarlet, congo, benzopurpurin):

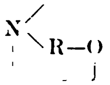

in the rosanilins (malachite green, brilliant green, methyl violet, methyl green, fuchsin, acid fuchsin, iodine green, aniline blue, alkali blue, water blue, aldehyde green).

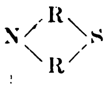

in the rosolic acid dyes (aurins).

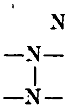

in the phthaleins (eosin, spriteosin, erythrosin, phloxin, rose bengale, rhodamin, gallein, cerulein).

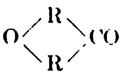

in the anthraquinones (alizarin, purpurin, anthragallol, alizarin blue).

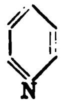

in the indamins (phenylene blue, Bindschedler's green, toluylene blue).


in the indophenols (indophenol blue).


in the thiazins (Lauth's dyes); (Lauth's violet or thionin, methylene blue, methylene red, methylene green).


in the azins (eurhodin, eurhodol, toluylene red, the safranins, Magdala red, mauvein).


in euxanthinic acid and possibly in galloflavin (jaune indienne).


in the quinolins and acridins (cyanin, quinolin red, quinolin yellow, acridin red, and scarlet).

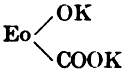
The majority of the aniline dyes are found in the market in the form of salts of the respective staining acids and bases, and it is noteworthy that the latter as such are for the most part either colorless or but feebly stained. Triaminotriphenylcarbinol is thus colorless, while its monacid salts are red (fuchsin); phenolphthalein likewise is colorless, but forms red salts with the alkalies; fluorescein is pale yellow, but forms the bright-red, fluorescent uranin with alkali, etc. The phenols and nitrophenols, however, are commonly used as free acids.

During the process of staining the salts of the staining acids or bases are probably decomposed by the animal or vegetable tissue and new compounds result between the free staining acid or base and the various chemical components of the tissue in accordance with the reaction of its component parts. The acid nuclear substance of cells thus shows a special affinity for basic dyes, and the basic protoplasm for acid dyes. Contrasted with this chemical process of staining is the physical process in which the dye is merely stored in the pores of the tissue. Both must be sharply differentiated the one from the other in attempting to draw inferences in reference to chemical affinity on the part of component parts of a tissue or a cell.

While in former years simple dyes were commonly employed in the clinical laboratory and tissues were stained *successively* if more than one dye was used, it has been shown that it is possible to combine acid dyes with basic dyes in such manner that the acid and basic affinities become more or less completely satisfied. The resulting compounds are spoken of as *neutral dyes*. In these the staining principles of the original components are preserved and in addition such compounds may show new staining properties which are dependent upon the union of the component dyes. They are accordingly termed *polychrome dyes*.

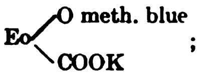
The credit of having first prepared such neutral dyes belongs to Ehrlich, whose triacid stain was for many years used almost exclusively in the clinical laboratory.

A well-known representative of the so-called neutral dyes is the eosinate of methylene blue. Eosin is a dibasic acid and can be represented by the formula



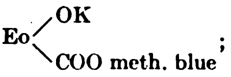
$$\mathrm{Eo}\begin{cases}\mathrm{OK}\\ \mathrm{COOK}\end{cases}$$

Three compounds with methylene blue thus appear possible, viz.:



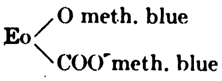
$$\mathrm{Eo}\begin{cases}\mathrm{O\ meth.\ blue}\\ \mathrm{COOK}\end{cases};$$

I



$$\mathrm{Eo}\begin{cases}\mathrm{OK}\\ \mathrm{COO\ meth.\ blue}\end{cases};$$

II



$$\mathrm{Eo}\begin{cases}\mathrm{O\ meth.\ blue}\\ \mathrm{COO\ meth.\ blue}\end{cases}$$

III

Although the dye has not been analyzed it is thought that formula I or II expresses its constitution. It would thus not be a true neutral dye, but a monacid salt. As a matter of fact other so-called neutral dyes are strictly speaking not neutral. Ehrlich's triacid stain is so called because it was assumed that the three basic radicles of the methyl green were all satisfied by the corresponding acid radicles of acid fuchsin and orange G. The existence of such a triacid salt is, however, impossible in aqueous solutions, even if it could occur theoretically, which in itself is impossible, as methyl green can only form triacid salts with concentrated mineral acids.

Practically important is the fact that two solutions of neutral mixtures can be directly mixed if they have one component in common, as in the case of Ehrlich's triacid stain, where methyl green is the common component.

While the simple dyes, both basic and acid, are soluble in water, the neutral dyes are practically insoluble, but soluble in an excess of either the acid or the basic component, and more especially the former. If then an aqueous solution of methyl green is added carefully to an aqueous solution of acid fuchsin, fuchsinate of methyl green is formed at once, but at first remains in solution owing to an excess of the acid dye. Upon the further addition of methyl green, however, a point is reached when the fuchsinate separates out, and if the amounts of the two components have been carefully determined beforehand the filtrate may be nearly colorless. If then an excess of methyl green is added, a certain amount of the fuchsinate will redissolve; and if the excess be sufficiently great, the entire precipitate will pass into solution.

Aside from an excess of the acid or basic component of the neutral dye its solution can also be brought about in other ways, as with alcohol (notably methyl alcohol), acetone, methylal, etc.

Not all simple dyes are equally well adapted for the preparation of neutral dyes. Of basic dyes, the most useful are those which contain the so-called ammonium group, notably methyl green, methylene blue, amethyst blue, and to a certain extent also pyronin and rhodamin; of acid dyes, the readily soluble salts of the polysulphonic acids, such as orange G, acid fuchsin, and narcein, and of the salts of the carbonic acid eosin.

Neutral mixtures may then be prepared which contain two or more component dyes. If it is desired to prepare a tricolor mixture two possibilities suggest themselves, viz., a mixture containing one acid dye and two basic dyes, or one with one basic dye and two acid dyes.

The principle of staining with neutral dyes is the same as in the case of the simple acid or basic dyes. Taking the leukocytes, for example, the nucleins will be found to decompose the neutral body and to unite with the basic component; the eosinophilic granules similarly decompose the dye, but take up the acid component, while in the case of the neutrophilic granules we may imagine that no decomposition is effected, but that the neutrophilic material unites directly with the neutral molecule.

Of the large number of staining mixtures which have been introduced within recent years, and of which many are mere modifications the one of another, I have placed only a small number of the more common ones before the reader at this place, and those only which personal experience has taught me to be useful and reliable. Where special mixtures are required in special work, they will be found described in their proper connection.

For routine work I should suggest Jenner's method or one of the Romanowsky modifications as described below, notably Giemsa and Hastings and Goldhorn. Ehrlich's triacid stain is retained in this edition because it is still used as a routine stain in some laboratories. It is largely of historical interest, however, and less valuable than the others which are mentioned.

Methods of Staining.

General Methods. **The Eosinate of Methylene Blue** (Jenner).[1]—Equal parts of a 1.2 to 1.25 per cent. *aqueous* solution of eosin and a 1 per cent. *aqueous* solution of methylene blue are mixed in an open basin and allowed to stand for twenty-four hours. The resulting precipitate—the eosinate of methylene blue—is washed with water, collected on a filter, dried at a moderate temperature, and finely powdered.[2] The dye can then be stored in bottles and is perfectly stable. For staining purposes a 0.5 per cent. solution in *absolute methyl alcohol* is employed; this can be used at once and keeps indefinitely. I have used this stain as a routine stain for years and can speak definitely of its value.

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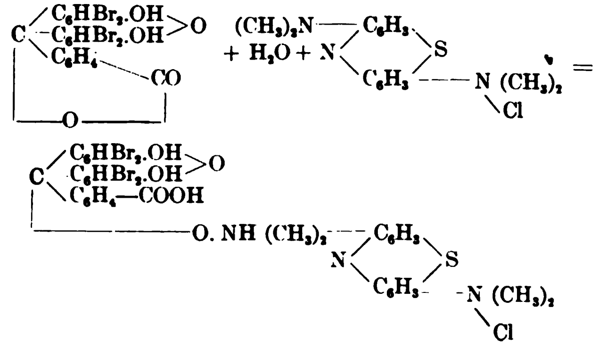
[1] Lancet, 1899, vol. i, p. 370. Simon, Maryland Med. Jour., April, 1900.

[2] The reaction probably takes place according to the formula:



In preparing the dye I first weigh out the requisite amount of eosin and methylene blue. The eosin is placed in a mortar or evaporating dish and rubbed into a paste with a small amount of water; more water is then added until all the dye is well dissolved. This solution is poured into a large saucepan and diluted to the proper point. The methylene blue is now similarly brought into solution, though with a little more difficulty, as the dye is inclined to be lumpy; it must all be dissolved. This solution is poured directly into the eosin solution and the requisite amount of water further added. The mixture is stirred with a rod and left to stand for twenty-four hours.

If the proper quantities have been used and well dissolved, the filtrate is but little colored, in which case not much washing is necessary; if, however, there is a distinct excess of either dye this must be washed out The precipitate is dried at a temperature not exceeding 60° C., and is then powdered. The alcoholic solution finally is prepared by rubbing up the dye with the alcohol in a porcelain dish. *Absolute methyl alcohol must be used.*

The blood films (on slides), which must be prepared without any pressure (the spreading slide should really be in contact only with the blood and not with the underlying slide), are not fixed before staining; this is accomplished by the absolute alcohol during the staining. The specimens are well covered with the stain and after about three minutes washed off with water and dried in the air. Care should be had during the staining that the preparations are thoroughly covered with the dye, as otherwise some of the stain is apt to become precipitated as the result of evaporation. After drying, the specimens can be examined directly in a drop of cedar oil. With the precautions stated, and by strictly adhering to the method as described, even the beginner can obtain perfect results. For routine purposes I can recommend the stain without reserve. The differentiation is excellent and most extensive (see Plates III, IV, and VI). The red corpuscles are stained a grayish terra cotta, the nuclei of the leukocytes and nucleated red cells blue, the plaques mauve, the neutrophilic granules a purplish red, the eosinophilic granules bright red, and the mast-cell granules dark violet. Granular degeneration and polychromasia of the red cells is well shown (Plate III). Malarial organisms, bacteria, and filarias are stained blue.

The *May-Grünwald stain*, which is frequently referred to in the German literature, is essentially the same as Jenner's.[1]

Ehrlich's Triacid Stain.[2]—The preparation of a reliable triacid stain, according to Ehrlich, presupposes the use of chemically pure dyes, such as those prepared by the Actiengesellschaft für

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[1] Centralbl. f. inn. Med., 1902, No. 11, and Deutsch. Arch., vol. lxxix, Heft 5 und 6.

[2] Ehrlich-Lazarus, Die Anaemie, loc. cit.

Anilinfarbstoffe of Berlin. Saturated aqueous solutions of orange G, acid fuchsin, and methyl green are first prepared and allowed to clear by standing for at least one week. It is essential that these solutions should be perfectly clear, and it is well in measuring off the requisite quantities to remove the supernatant portion with a pipette.

The various components are then mixed in a clean bottle, making use of the same measuring glass, and without washing between the addition of the individual components. These are taken in succession as shown below, and after adding the methyl green the mixture is thoroughly stirred until the remaining portion of alcohol and glycerin has been added.

Orange G solution	13.0–14.0 c.c.
Acid fuchsin solution	6.0–7.0 c.c.
Distilled water	15.0 c.c.
Absolute alcohol	15.0 c.c
Methyl-green solution	12.5 c.c.
Absolute alcohol	10.0 c.c.
Glycerin	10.0 c.c.

The solution is ready for use at once and does not deteriorate with age.

In order to obtain the best results, it is practically necessary to fix the blood films by heat; fixation by Nikiforoff's method does not furnish constant results, and only too often leaves the neutrophilic granules unstained or imperfectly stained. Fixation at a high temperature (140° C.), as suggested by Rubinstein, furnishes better results than the lower temperatures originally advised by Ehrlich, as the difference in color between the neutrophilic granules and the eosinophilic granules is brought out more prominently. The blood specimens are stained about five minutes, then washed in water, dried (by blotting, if desired), and examined as usual.

In properly stained specimens the eosinophilic granules present a copper or a yellowish-red color, while the neutrophilic granules are violet. The mast-cell granules remain colorless and appear as round vacuoles in the faintly bluish-green protoplasm. The nuclei of the leukocytes present a greenish color and are not well stained. The red cells in properly heated specimens are orange; if the temperature was too high they are yellow, and it will be found that their structure has suffered as a consequence. If the temperature has been too low the red cells take on the fuchsin. The nuclei of the normoblasts are intensely stained; the older nuclei appear black; megaloblastic nuclei, on the other hand, are rather feebly stained, and in some specimens, indeed, the inexperienced will at first sight not discern any nucleus. Granular degeneration is not shown and polychromatophilia cannot be well demonstrated. Malarial organisms are imperfectly shown. The differentiation with the triacid is thus markedly less than in the case of the eosinate. This is owing to the peculiar character of the methyl green, which is a specific nuclear dye. To counteract some of these deficiencies, Ehrlich has suggested to stain the preparations for a few seconds with an aqueous solution of methylene blue first, and to stain with the triacid afterward. This improves the pictures somewhat, but it is not wholly satisfactory.

The Romanowsky Method.[1]—The history of the Romanowsky method is intimately associated with the study of the minute structure of the malarial organism, in which the presence of a nucleus was first demonstrated by its aid. The dye is essentially an eosin-methylene-blue mixture, the specific staining action of which is, however, not due to the methylene blue *per se,* but to a decomposition product of the methylene blue, viz., methylene azure.[2] This apparently combines with eosin to form a neutral dye analogous to the eosinate of methylene blue, and can similarly be used as a routine blood stain in the clinical laboratory. As a rule we do not employ solutions of the pure dye, however, but solutions of methylene blue containing a variable amount of the methylene azure, to which the requisite amount of eosin is added.

The following modifications of the original Romanowsky method are based in principle upon the above considerations:

Hastings' Method.[3]—Three solutions are prepared, viz., (1) a 1 per cent. aqueous solution of eosin (Grübler's water soluble, yellow shade); (2) a 1 per cent. aqueous solution of methylene blue (Ehrlich's rectified), and (3) a solution of polychrome methylene blue.

The polychrome methylene-blue solution is made according to the formula: methylene blue (Ehrlich's rectified) 2 grams, sodium carbonate (dry powder) 2 grams, distilled water 200 c.c. The carbonate is dissolved in hot distilled water and the methylene blue rubbed up in the proportion indicated. The solution is boiled over a free flame or kept on a boiling water bath for ten to fifteen minutes; 30 to 40 c.c. of water are added for each 100 c.c. to allow for evaporation. The boiling is continued for ten to fifteen minutes longer. The hot solution is poured off from the sediment, and if necessary brought to the 200 c.c. mark by diluting with distilled water, after which it is partially neutralized with dilute acetic acid (12.5 to 20 per cent.

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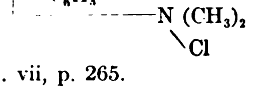
[1] St. Petersburg. med. Woch., 1891, and Nocht, Encyk. d. mik. Tech., vol. ii, Urban u. Schwarzenberg, Berlin-Wien, 1903, p 785.

[2] Methylene azure is an amphoteric dye, *i. e.*, a dye of basic constitution with acid properties; it is the sulphone of methylene blue and has the formulas:



[3] Jour. Exper. Med., vol. vii, p. 265.

solution). Hastings points out that it is well to add the acetic acid to one-half of the polychrome-blue solution until a well-marked acid reaction to litmus paper is obtained (6 or 7 c.c. of 12.5 per cent. acid, or 3 or 4 c.c. of the 20 per cent. acid to 100 c.c.) and to mix this neutralized portion with the other half, so as to prevent overneutralization. The solution should be alkaline in final reaction, since a slight excess of acid destroys the polychrome properties, which cannot be restored by the addition of alkalies.

The three solutions are then mixed in the following proportion and in the following order:

Distilled water	1000 c.c.
1 per cent. eosin solution	100 c.c.
Polychrome-blue solution	200 c.c.
1 per cent. methylene-blue solution	70 c.c.

The mixture is stirred. A green, metallic-looking scum appears on the surface and a fine precipitate separates out. To bring this about it may be necessary to add a little more of the 1 per cent. methylene-blue solution, viz., 80 instead of 70 c.c.

The mixture may be filtered at once or after standing for twenty to thirty minutes. The residue is allowed to dry in the air or in the drying oven at a temperature not above 60° C. It is finally pulverized and can be stored in this form. The amounts of the dyes indicated above furnish from 0.7 to 1 gram of the ultimate product.

For staining purposes a 0.25 per cent. solution in *absolute methyl alcohol* is used, which is prepared by rubbing up the dye with the alcohol in a mortar. If successful the solution has a purple plum color.

Care should be had that the alcohol is neutral. Some lots of methyl alcohol show an acidity of 1 to 2 c.c. of $\frac{n}{10}$ alkali for 100 c.c. Such specimens must be neutralized by the addition of 0.05 to 0.1 gram of dry sodium carbonate for 100 c.c.

Previous fixation of the blood specimens is not necessary, as the alcohol fixes while the staining is going on. The films are covered with the solution and left for one minute, after which they are differentiated by the addition of water until a greenish, metallic-looking scum appears on the surface (15 drops to a slide). This is continued for five minutes, when the preparations are rinsed for two to three seconds in water and immediately dried by blotting. This procedure will answer for all ordinary purposes, and for bringing out the young forms of the malarial parasite, but for the maturer forms it is better to stain for two minutes and to differentiate for ten.

The negative surface of the specimen should be carefully inspected and washed if necessary, to remove any dried stain that may be present and which appears as a thick, greenish coating.

In a properly stained specimen the red cells appear red; in over-stained or old specimens light gray or light blue. Polychromatophilia and granular degeneration are well shown. The neutrophilic granules are bright red, the eosinophilic granules eosin colored, and the mast-cell granules dark red. The nuclei of the lymphocytes, large mononuclear leukocytes, and myelocytes are magenta red; those of the polynuclear leukocytes a bluish violet. In some of the lymphocytes and large mononuclear leukocytes Michaelis' granules will be seen. The blood plates are pale blue with red nuclei. The nuclei of the red blood corpuscles are red. The malarial organisms present a blue body with one or more intensely red nuclear structures, varying in size from that of a tiny dot in the youngest forms to a structure which in the microgametocytes fills the entire body of the parasite in the form of a fine reticulum. In the segmenting bodies each segment contains a red nucleus, while the body is blue.

LEISHMAN'S METHOD.[1]—Leishman also makes use of the isolated eosinate of methylene blue mixed with eosinate of methylene azure. He proceeds as follows: Two solutions are prepared: one a 1 pro mille solution of eosin (Grübler's extra B. A.) in distilled water; the other a 1 per cent. solution of medicinal methylene blue (Grübler), also in distilled water, and alkalinized with sodium carbonate to the extent of 0.5 per cent. This last solution is heated to 65° C. for twelve hours, and is then allowed to stand at the temperature of the room for ten days before using. Equal volumes of the two solutions are mixed in a large open basin and allowed to stand for from six to twelve hours, the mixture being stirred from time to time with a glass rod. The resulting precipitate is collected on a filter, thoroughly washed with distilled water, dried, and powdered. A 0.15 per cent. solution of the dye in pure methyl alcohol serves as stain and does not deteriorate on keeping. Special fixation is not required. The blood film is covered with the solution and stained for about one-half minute. Double the amount of distilled water is then added and allowed to mix with the alcoholic solution. After five to ten minutes the stain is washed off with distilled water, a few drops of water being allowed to rest on the film for a minute. The specimen is next dried (without heat) and can be examined as usual. The soaking in water for a minute after the staining is important, as it intensifies the Romanowsky stain; it changes the tint of the red corpuscles from a greenish-blue to a transparent pink or greenish color, while the nuclei of the leukocytes are usually a ruby red. The nuclei of nucleated red cells are almost black and the extranuclear portion gray. The blood plates are a deep ruby red with shaggy margins, frequently showing a pale-blue peripheral zone surrounding the red centre. The body of the malarial parasite stains blue and its chromatin a ruby red. In the case of the tertian parasite Schüffner's dots are well marked in the containing red corpuscles.

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[1] Brit. Med. Jour., September 21, 1901.

WRIGHT'S MODIFICATION OF LEISHMAN'S METHOD.[1]—Wright has simplified Leishman's method in several important particulars, which render it even more convenient for routine work; he has ascertained, moreover, that any one of the Grübler methylene blues can be employed for the purpose of obtaining a sufficient quantity of methylene azure.

The staining fluid is prepared as follows: 1 per cent. of methylene blue is added to a 1 per cent. aqueous solution of sodium bicarbonate, when the mixture is steamed in an Arnold steam sterilizer for one hour. On cooling, the solution is poured directly into a large dish or flask and treated, while stirring or shaking, with a sufficient quantity of a 1 pro mille solution of eosin (yellow shade) until the mixture has assumed a purple color and a scum with a metallic lustre forms on the surface. This will require about 500 c.c. of the eosin solution for 100 c.c. of the methylene-blue solution. The resultant precipitate, which contains both eosinate of methylene blue and eosinate of methylene.azure, is collected on a filter, and without further washing allowed to dry. When thoroughly dry, a 0.3 per cent. solution in *pure* methyl alcohol is prepared (this is practically a saturated solution). The solution is filtered and to the filtrate 25 per cent. methyl alcohol further added so as to dilute the stain somewhat and to lessen the tendency of the dye to become precipitated during the process of staining.

The air-dry blood films are covered with the stain for one minute; water is then added drop by drop until the staining fluid becomes semitranslucent and a reddish tint becomes visible at the margins, while a scum with a metallic lustre forms on the surface. The amount of water required will vary with the amount of staining fluid on the preparation, but in general it may be said that 8 or 10 drops will suffice if a seven-eighths inch square cover-glass is used. The staining fluid, thus diluted, is allowed to remain on the preparation for two or three minutes, during which time the real staining of the specimen takes place. It is then washed off, when the blood film will be seen to have a blue or purple color.

The next step is to develop the differential staining of the various elements in the preparation. This is done by washing the preparation in water, preferably distilled water, until the better-spread portions of the film appear yellowish or reddish in color. If desired, the process of differentiation may be readily observed by placing the cover-glass, film side uppermost, on a slide, covering it with water, and examining it with the microscope under a low magnifying power. The red blood corpuscles, which, as before stated, at first have a blue color, will become greenish, then yellowish, and finally orange or pinkish in color, depending upon the depth of the original staining, which varies with the length of time that the diluted staining fluid has been allowed to act, and with the degree of its dilution.

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[1] Jour. Med. Research, 1902, vol. vii.

The differentiation by washing in water seems to be essentially a process of decolorization by which some of the blue constituent of the dye is removed, for the water that drains off from the preparation has a blue color. This differentiation or decolorization proceeds slowly, and may require from one to three minutes, depending upon the intensity of the staining and upon the tint sought to be obtained in the red corpuscles.

It is apparent from the above that with a little experience with the method the color of the red corpuscles may be made either orange or pink. When the desired color is obtained in the red corpuscles the preparation is quickly dried between layers of filter paper and mounted in balsam. It is important to arrest the decolorization by drying the preparation as soon as the desired tint in the red corpsucles is obtained, for it may be carried too far.

Dried blood films may be kept for weeks without impairment of their staining properties. Films months old will probably not give good results.

In a suitably stained specimen the red cells are either orange or pink; polychromatophilia and granular degeneration are well shown (the granules blue); the neutrophilic granules are a reddish lilac; the eosinophilic granules eosin colored; the mast-cell granules a dark blue, a dark purple, or even black. The lymphocytes have dark purplish-blue nuclei with robin's egg blue protoplasm, in which the granules described by Michaelis appear dark blue or purplish. The large mononuclear leukocytes present a blue or dark lilac colored nucleus, and in some Michaelis' granules can also be made out. The blood plates are stained a deep blue or purplish and the malarial organisms are colored as with Leishman's method.

Giemsa's Method.[1]—Giemsa's stain has the following composition:

Azure II (azure plus methylene blue āā)	3.0
Eosin (B. A.)	0.8
Glycerin (Merck, C. P.)	250.0
Methyl alcohol (Kahlbaum I)	250.0

It is prepared by grinding up the dyes in the absolute alcohol and then adding the glycerin. The blood films are fixed for a minute in absolute methyl alcohol and then stained for five minutes in a mixture of 14 drops of the dye to 10 c.c. of distilled water, which is always freshly prepared; a trace of sodium carbonate may be added to the water to intensify the basic colors. After washing in water the films are blotted and are then ready for examination. The various elements are stained as with the methods already described.

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[1] Centralbl. f. Bakter. Abt. I, vol. xxxvii, 2, p. 308

Goldhorn's Method.[1]—The blood smears are fixed with pure methyl alcohol for fifteen seconds, washed in running water, stained for thirty seconds in a 1 per cent. aqueous solution of eosin, washed, stained for one minute in Goldhorn's polychrome methylene blue, again washed and dried in the air.

The polychrome methylene blue is prepared as follows: 2 grams of methylene blue and 4 grams of lithium carbonate are dissolved in 300 c.c. of warm water. The solution is heated in a porcelain dish on a boiling water bath for 15 minutes, then poured into a glass-stoppered bottle and set aside for several days. Finally it is rendered only slightly alkaline by the careful addition of 4 to 5 per cent. acetic acid solution (test with litmus paper). The method gives excellent results.

DEMONSTRATION OF IODOPHILIA.

Cover-glass specimens are prepared as usual; after drying in the air they are placed in a small jar containing a few crystals of iodine. After several minutes the films assume a dark-brown color, when they are mounted in a drop of a saturated solution of levulose and examined with an oil-immersion lens. The red corpuscles are stained light yellow, while the leukocytes are almost colorless. All glycogen granules, whether contained in leukocytes or free in the blood, are stained a distinct mahogany.

This method furnishes better results than the older method of staining with a solution composed of 1 gram of iodine and 3 grams of potassium iodide in 100 grams of a concentrated solution of mucilage (1 part of Lugol's solution to 100 parts of a thick mucilage.[2]

ENUMERATION OF THE CORPUSCLES OF THE BLOOD.

Method of Thoma (Author's Modification).[3]—The instrument consists of two diluting pipettes and a counting chamber (Fig. 19). The latter is ruled into 100 large squares (*A*, *A*, *A*), each occupying an area of $\frac{1}{25}$ sq. mm. (Fig. 20). They are separated from one another by double guiding lines (*a b*, *a b*) with an intervening distance of $\frac{1}{20}$ mm. Where the horizontal and vertical lines intersect small squares (*a*, *a*, *a*) result, 100 in number, which accordingly have an area of $\frac{1}{400}$ sq. mm. each. The large squares are thus bounded by rectangles (*b*, *b*, *b*), measuring $\frac{1}{20}$ mm. in width by $\frac{4}{20}$ mm. in length, representing an area of $\frac{1}{100}$ sq. mm.

As the little platform (*f*) carrying the ruling is exactly $\frac{1}{10}$ mm.

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[1] The New York Univ. Bull. of Med. Sci., 1901, vol. i, No. 2.

[2] Ehrlich-Lazarus, Die Anaemie, loc. cit.

[3] The counting chamber can be procured from Ernst Leitz & Co., New York.

lower than the outside glass plate (*e*), each large square represents the base of a cube the contents of which are $\frac{1}{25} \times \frac{1}{10} = \frac{1}{250}$ cb. mm.; each small square similarly corresponds to $\frac{1}{400} \times \frac{1}{10} = \frac{1}{4000}$ cb. mm., and each rectangle to $\frac{1}{100} \times \frac{1}{10} = \frac{1}{1000}$ cb. mm.

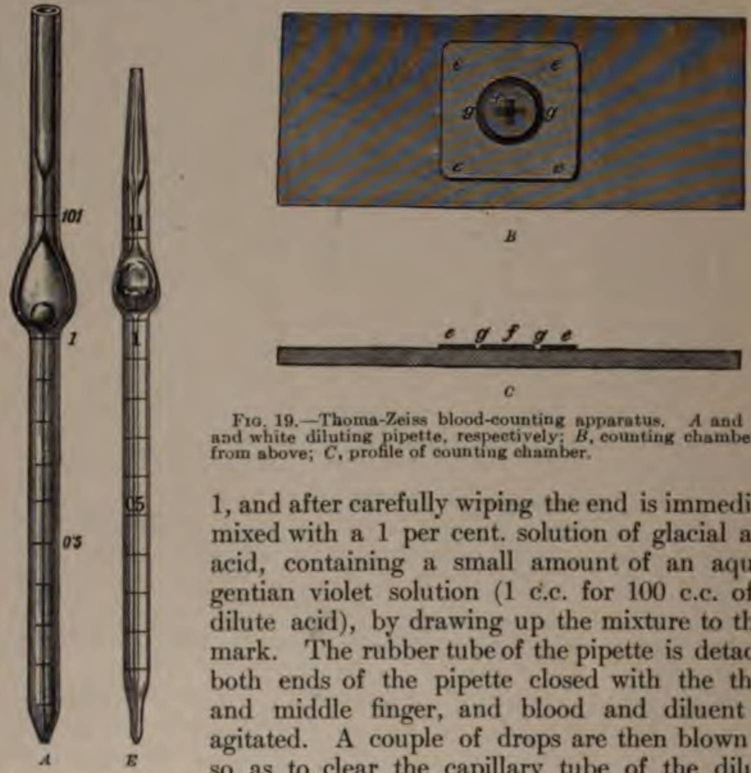

Fig. 19.—Thoma-Zeiss blood-counting apparatus. *A* and *E*, red and white diluting pipette, respectively; *B*, counting chamber, seen from above; *C*, profile of counting chamber.

1. **Enumeration of the Leukocytes.**—A drop of blood is procured by freely puncturing the finger or the lobe of the ear, after cleaning and drying the skin, wiping away the first drop or two, and avoiding undue pressure. It is drawn into the 1 to 10 diluting pipette to the mark 1, and after carefully wiping the end is immediately mixed with a 1 per cent. solution of glacial acetic acid, containing a small amount of an aqueous gentian violet solution (1 c.c. for 100 c.c. of the dilute acid), by drawing up the mixture to the 11 mark. The rubber tube of the pipette is detached, both ends of the pipette closed with the thumb and middle finger, and blood and diluent well agitated. A couple of drops are then blown out, so as to clear the capillary tube of the diluting fluid which has not entered the bulb of the pipette. A drop of the diluted blood is now placed upon the platform of the counting slide, and one of the cover-glasses which accompany the instrument adjusted in such a way as to exclude bubbles of air. The size of the drop should be such that, when the cover-glass is in place, it does not run over into the moat (*g*) surrounding the circular platform, nor even project over the sides. Türk advises that a *tiny* droplet of the pure diluting fluid be placed upon the plate *D*, before the diluted blood is placed upon the counting platform. If cover and slide have been previously scrupulously cleansed and slight pressure is now made upon the cover where it overlies the plate *D*, Newton's

There are in
cells.

colored rings will become visible—a sign that a successful mount has been made. The slide is set aside for a few minutes, so that the corpuscles settle down, when it is examined with a high power (Leitz Ocular 2 and Obj. 6).[1] It will be noted that the red corpuscles are invisible and that the leukocytes are colored blue. For counting a mechanical stage is very convenient. Starting with the top row of large squares at the left corner, the total number of leukocytes in the 100 large squares is now carefully counted. This number divided by 100 gives the average number of leukocytes for *one* large square. As the cubic contents of each large square are $\frac{1}{250}$ cb. mm., it is only necessary to multiply the number of leukocytes in one square by 250 in order to find the number for 1 cb. mm. of *diluted* blood, and this by the degree of dilution (in the above instance by 10) to find the number for 1 cb. mm. of diluted blood, and 1000 × 10 the number in 1 cb. mm. of non-diluted blood.

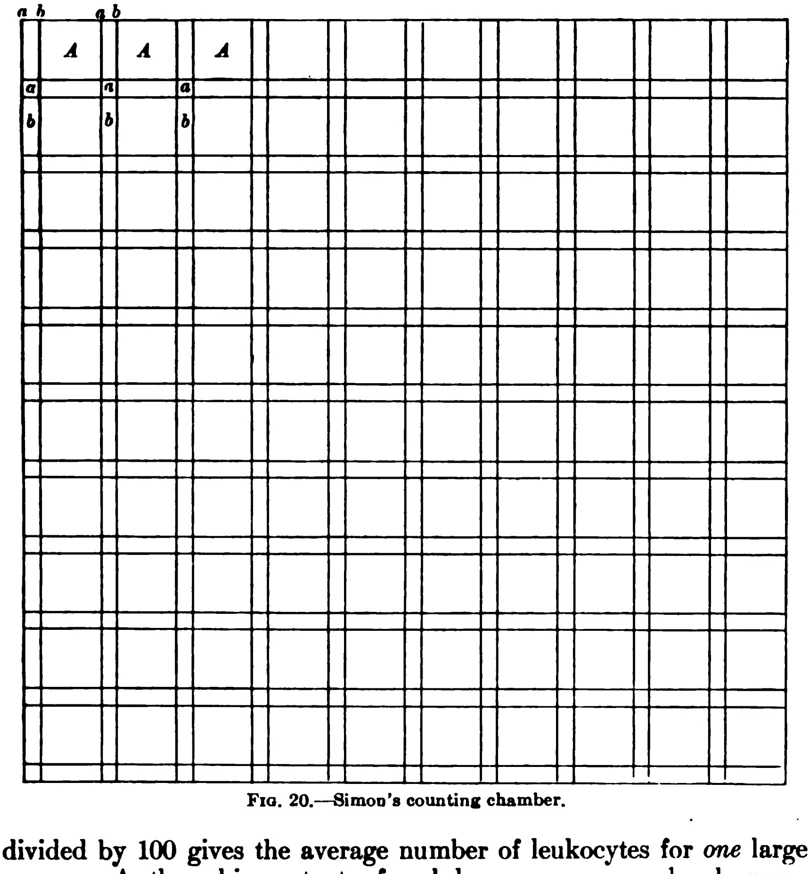

Fig. 20.—Simon's counting chamber.

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[1] To focus through the thick cover the objective should have a long working distance.

EXAMPLE.—Total number of leukocytes counted in the 100 large squares = 400; hence $\frac{400}{100}$, viz., 4 = number of leukocytes in a single square, *i. e.*, in $\frac{1}{250}$ cb. mm. of diluted blood; hence 250 × 4 = 1000, the number of leukocytes in 1 cb. mm. of non-diluted blood, and 1000 × 10 the number in cb. mm. of non-diluted blood.

When counting the cells note should only be taken of such that lie within the squares or upon the upper and left boundary lines; cells upon the right and lower lines should be omitted.

In the above instance a dilution of 1 to 10 has been advocated. This may be used as a matter of routine. If a marked grade of leukocytosis is anticipated a dilution of 1 to 20 will be found more convenient.

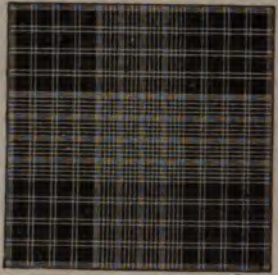

FIG. 21.—Türk.

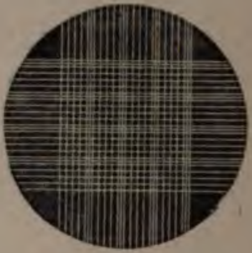

FIG. 22.—Thoma; centre part.

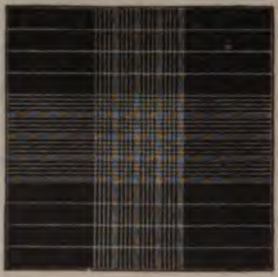

FIG. 23.—Zappert-Ewing.

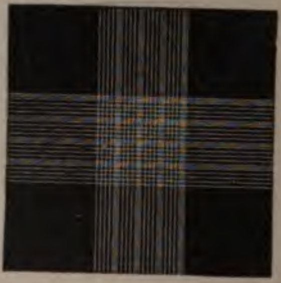

FIG. 24.—Thoma.

Blood-counting chambers.

If desired even higher dilutions may be used, in which case the red pipette permitting of a dilution of 1 to 100 or more is employed.

2. **Enumeration of the Red Cells.**—The blood is diluted 100 times by filling the red pipette with blood to the mark 1 and with the diluent to 101. For diluting the blood in the enumeration of the red corpuscles Toison's solution is most convenient:

Sodium chloride	1.0
Sodium sulphate	8.0
Neutral glycerin	30.0
Distilled water	160.0
Methyl violet (5 B.)	0.025

To prevent the development of molds the solution should further contain about 1 pro mille of thymol.

After mixing the diluent and blood thoroughly and blowing out the pure diluting fluid in the capillary tube a drop is mounted as described. All the red corpuscles are then counted—in the 100 *small* squares, if no marked degree of anemia exists, or in 40 or more *rectangles* if the corpuscles are distinctly diminished. The calculation is then made as follows, bearing in mind the cubic contents, corresponding to the small square and the rectangle, viz., $\frac{1}{4000}$ and $\frac{1}{1000}$ cb. mm., respectively:

Example 1.—Number of red cells in 100 small squares = 1000; in 1 therefore 10, viz., in $\frac{1}{4000}$ cb. mm.; in 1 cb. mm. of diluted blood 4000 × 10 = 40,000 and in 1 cb. mm. of non-diluted blood 40,000 × 100 = 4,000,000.

Example 2.—Number of red cells in 40 rectangles = 800; in 1 reactangle therefore $\frac{800}{40} = 20$, *i. e.*, in $\frac{1}{1000}$ cb. mm.; in 1 cb. mm. of diluted blood hence 20 × 1000 = 20,000, and in 1 cb. mm. of non-diluted blood 20,000 × 100 = 2,000,000.

If for any reason a larger area is to be counted for red cells, this can, of course, be readily done by going over a larger number of rectangles, or by combining small squares and rectangles, due allowance being made for the cubic contents of the ground covered.

Other counting chambers are also in existence. The form of the ruling of various models is shown in the accompanying figure (Figs. 21 to 24). They are used in the same manner as that of the author. The calculation in each case depends upon the number of squares counted, and its corresponding cubic contents and the degree of dilution.

Second Method.—If a counting chamber with one of the more modern rulings is not available, but if a mechanical stage is at hand, the leukocytes can also be counted with the old Thoma counter in the following manner: A drop of the diluted blood is mounted as usual. With the mechanical stage a field corresponding to the position of 1 in the accompanying diagram (Fig. 25) is then selected as the starting point. The presence or absence of leukocytes is noted and the field changed, so that an adjoining circle is brought into view, and so on. In this manner at least 100 circles are gone over, using a corpuscle to the side or above or below as a guide to the next field. The total number of leukocytes is noted and the average for one circle calculated. If the cubic contents corresponding to each circle are known, the calculation of the number of leukocytes in 1 cb. mm. of blood becomes a simple matter. The determination of the cubic contents corresponding to a circle is made as follows: Noting the number of the eyepiece and the objective, the diameter of the field of vision is measured with a stage micrometer, or with the aid of the rulings of an ordinary Thoma-Zeiss counter, bearing in mind in the latter case that the distance between two vertical lines is $\frac{1}{20}$ mm. The area of the circle, according to geometrical law, will then be equivalent to $\pi\rho^2$, in which π is a constant factor—*i. e.*, 3.1416; and ρ the radius, from which the corresponding cubic contents are calculated by multiplying the result by 0.1—*i. e.*, the depth of the counting chamber. The resultant value, which should be ascertained for every instrument separately, will, of course, be constant for the system of lenses and the counting chamber used. With a Bausch & Lomb $\frac{1}{6}$ (long-working distance), the 1-inch eyepiece, and 160 mm. tube length, the cubic contents of the field are 0.009 cb. mm.

Example.—The blood was diluted 100 times. In 100 fields 50 leukocytes were noted—*i. e.*, 0.5 for 1 field, or for 0.009 cb. mm.; in 1 cb. mm. of diluted blood there would hence be 0.5 divided by 0.009 = 55.5, and for 1 cb. mm. of undiluted blood 55.5 × 100 = 5550 leukocytes.

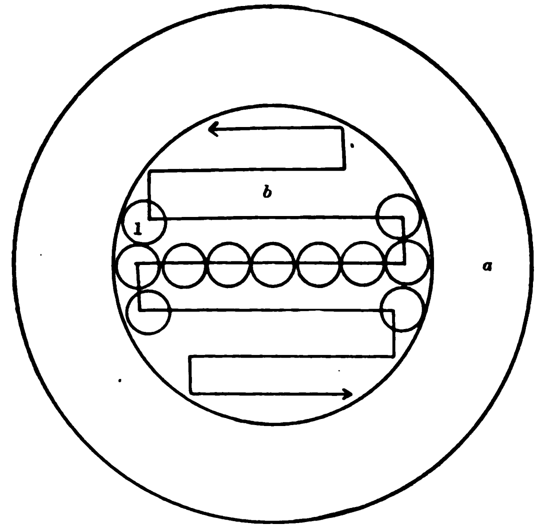

Fig. 25.—Schema of circles for counting leukocytes: *a*, moat surrounding central platform, *b*, of counter; 1, starting point.

Cleaning of the Apparatus.—After use the apparatus must be carefully cleansed. The pipette is washed out with the diluting fluid, then with water, next with absolute alcohol, and finally with ether. The washing will be facilitated by slipping the rubber tube over the long arm of the pipette and blowing the contents of the bulb out of the short arm. In laboratories which are equipped with a suction pump this may be conveniently employed; the entire process then occupies only two or three minutes.

The counting chamber is washed with water only; alcohol and ether dissolve the substance with which the platform is cemented to the slide.

Differential Enumeration of the Leukocytes.—The differential enumeration of the leukocytes is usually made in dried and stained specimens. A mechanical stage is a great convenience, but not a necessity. The idea is to go over a large number of cells, for ordinary purposes not less than 500 to 600, to classify these, and finally to calculate the percentages. The cells are charted as shown below:

S. M. (small mononuclear leukocytes): 卌 卌 卌 卌
卌 卌 卌 卌 卌 = 45
L. M. and T. F. (large mononuclear leukocytes and transition
forms): 卌 卌 卌 = 15
P. (polynuclear neutrophiles): 卌 卌 卌 卌 卌
卌 卌 卌 卌 卌 卌 卌 卌 卌 卌
卌 卌 卌 卌 卌 卌 卌 卌 卌 卌
卌 卌 卌 卌 卌 卌 =155
E. (eosinophiles): 卌 = 5
M. (mast-cells): // = 2

222

Result: Total number of cells counted, 222, of which:

small monos., $\frac{45\times 100}{222} = 20.2$ per cent.

large monos., $\frac{15\times 100}{222} = 6.7$ "

polys., $\frac{155\times 100}{222} = 69.8$ "

eosins., $\frac{5\times 100}{222} = 2.2$ "

mast., $\frac{2\times 100}{222} = 0.9$ "

While making a differential count it is always well to keep note of the time, as it is often possible in this way to form a fair idea of the *actual* number of the leukocytes without an absolute count. This, of course, requires a certain amount of experience in the preparation of the smears, which should be uniformly of nearly the same thickness. After one has then learned by control how many leukocytes in a blood smear, observed within a certain length of time, may be considered as normal, it is not difficult to judge the grade of a hyperleukocytosis by the increase in number noted within the same length of time. Everyone must here work out his personal equation. A general idea of the degree of increase can, of course, be formed by examining the specimen with a low power—a Bausch & Lomb $\frac{2}{3}$, for example—but in the manner indicated one gets a numerical expression which is at times quite helpful. The count itself can be readily made with a $\frac{2}{3}$; it requires a little practice, but proves a great saving of time.

Enumeration of the Plaques.—For this purpose the method of Broide and Russel has been advocated. The method is an indirect one. First, the red corpuscles are counted in the usual manner. A drop of the staining fluid, composed of equal parts of a 2 per cent. solution of common salt and a saturated solution of dahlia in glycerin, is then placed upon the finger, when this is punctured through the drop and the blood allowed to mix with the reagent. In this mixture the ratio between the plaques and the red corpuscles is ascertained, and the total number of plaques contained in 1 cb. mm. of blood determined by calculation. The plaques are stained the color of dahlia and can readily be counted. Rapid work is essential, as the staining fluid soon attacks the red corpuscles.

Other writers determine the ratio of plaques to red cells in smears and then calculate their number after an absolute red-cell count. Jenner's stain or any one of the methylene-azure mixtures (Hastings', Giemsa, Wright) will answer the purpose.

The Hematocrit.—The use of the hematocrit for counting the red blood corpuscles has been repeatedly advocated, but has not met with favor. The method is inapplicable whenever there is any material variation in the size and form of the red corpuscles and whenever the number of the leukocytes is greatly increased. This means that the method cannot be employed in the majority of cases in which we are especially interested in the blood count. If, however, it is desired to ascertain the volume of the red corpuscles in relation to the amount of plasma, the instrument will furnish satisfactory results. A centrifuge run by electricity is practically a necessity; in this way alone is it possible to maintain the proper rate and uniformity of speed. Hand centrifuges are, in my experience, totally inadequate, and with instruments driven by water power it is impossible to attain a sufficient rate of speed for this purpose. An apparatus like the one pictured in the accompanying illustration (Fig. 26) answers the purpose best. It is connected with the street current or with a small battery, a rheostat being interposed to control the current and the rate of speed. At the same time a speed indicator can be attached which strikes a bell for every 100 revolutions. For the hematocrit a speed of 8000 to 10,000 revolutions per minute is required.

The hematocrit which is almost exclusively used in the United States is that of Daland (Figs. 27, 28, 29). It consists of a metallic frame which carries two glass tubes measuring 50 mm. in length and 0.5 mm. in diameter. Each tube bears a scale ranging from 0 to 100, the individual divisions of which are rendered more easily visible by a magnifying lens front. In the frame the outer end of each tube fits into a small depression, the bottom of which is covered with thin rubber; the inner ends are held in position by springs. The instrument is screwed to a firm table and is oiled daily when in use.

If the patient is directly available, undiluted blood is used. The finger is washed with soap and water and alcohol, as usual, and is freely punctured. A small rubber tube is then slipped over the end of one of the hematocrit tubes, which is completely filled by suction. The bevelled end of the tube is quickly covered with the finger, which has been previously lubricated with a little vaselin; the rubber tube is disconnected, and the glass tube immediately fixed in the one compartment of the frame. Its mate is rapidly placed on the opposite side and the instrument rotated at a speed of from 8000 to 10,000 revolutions per minute for three minutes, when the volume is directly read off. In normal individuals the volume of the red corpuscles is approximately 50 per cent., so that in a given case a proportionate expression of the percentage of corpuscles, as compared with the normal, can be obtained by multiplying the figure on the scale by 2.

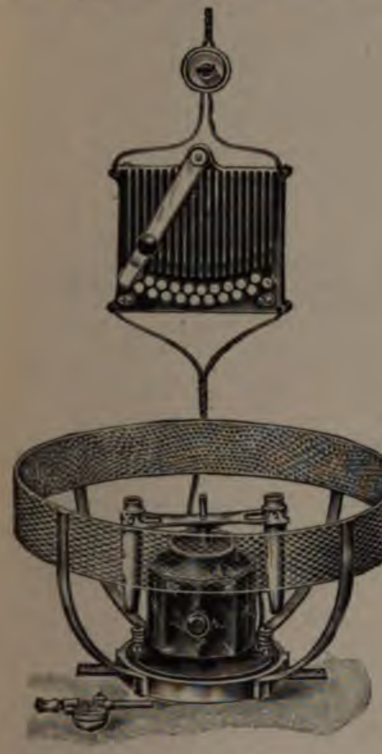

FIG. 26.—Improved electric hematocrit, with fender, rheostat, and speed indicator. The hematocrit attachment replaces the urine tubes seen in the revolving armature.

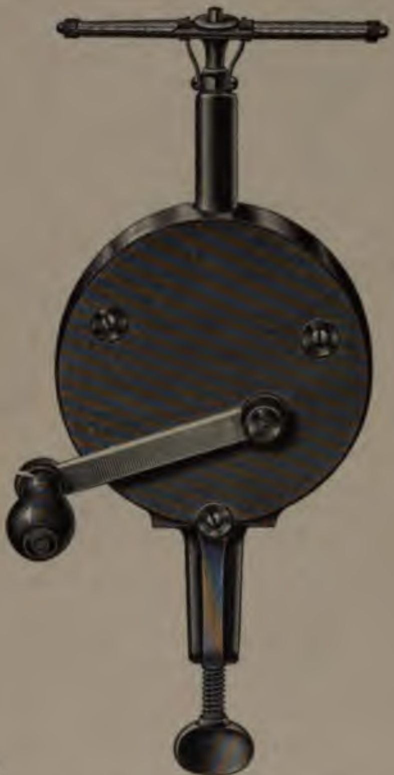

FIG. 27.—Daland's hematocrit.

If the patient is not directly available, the blood is diluted with an equal volume of a 2.5 per cent. solution of potassium bichromate, as proposed by Daland. As Ewing suggests, this can be done with the pipette which accompanies the Thoma-Zeiss blood counter. In the case of the red pipette the capillary tube is filled with blood to the mark 1, then a small air bubble is drawn in, followed by another tube length of blood. Three or four volumes of blood are obtained in this way and diluted at once with an equal quantity of the bichromate solution. In the case of the white pipette a single tube length of blood and the diluent is sufficient. Blood and diluent are thoroughly mixed, care being had not to include any air bubbles. In this form the blood is carried to the laboratory, where both tubes are filled by allowing the drops to flow in from the point of the pipette. To obtain the percentage volume the resultant figure is in this case, of course, multiplied by 4.

In the case of normal blood it has been ascertained that 1 per cent. by volume, as read off from the scale, corresponds to almost 100,000 red corpuscles per cb. mm.; to obtain the total number of red cells per cb. mm., it is hence only necessary to add five ciphers to the percentage indicated on the scale.

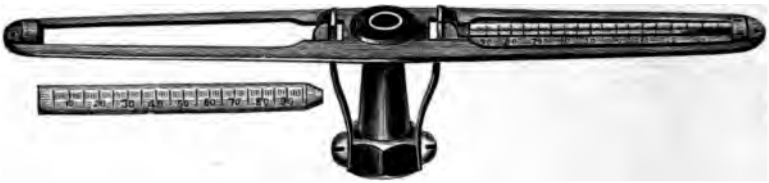

FIG. 28.—Daland's hematocrit.

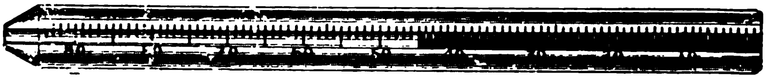

FIG. 29.—Daland's hematocrit tube.

Example.—Undiluted blood was used; the reading on the scale was 45. The volume per cent. of the red corpuscles would hence be 90, and the number of red cells per cb. mm. 4,500,000.

But, as I have pointed out, this calculation presupposes that the size and form of the red cells are practically normal, and that the leukocytes are not materially increased.

With normal blood the leukocytes appear only as a narrow, indistinct, milky band at the central end of the column of red cells, which with a material increase of the leukocytes becomes more marked and reaches its greatest extent in well-marked cases of leukemia.

Aspelin has recently suggested that with a suitable modification of the Daland apparatus quite accurate leukocyte counts can be obtained by centrifugation; but bearing in mind the variations in the size of the different leukocytes and the varying degree in which the different forms take part in the production of the different types of hyperleukocytosis, it is evident at once that still less is to be anticipated from the centrifugal method in this direction than in the case of the red cells.

Volume Index.—The term *volume index* has been introduced by Capps to designate the relation existing between the volume of red cells determined by centrifugation (see above) and their number. If both are normal the ratio $\frac{\text{volume (100 per cent.)}}{\text{number (100 per cent.)}} = 1$ (0.99 average of 10 normal individuals). In 29 cases of pernicious anemia the volume index was high during the active stage of the disease, ranging from 1.05 to 2.0. During periods of improvement it steadily fell, while in periods of decline it steadily rose. In chronic secondary anemia of moderate intensity normal values are the rule; in a few they are low. In acute secondary anemia (sepsis, hemorrhage) the index may be low (0.72); so also in chlorosis of the severer type. In a few cases of chronic severe secondary anemia (as in uncinariasis) Capps found the volume index high. Analogous results have been obtained by Wroth.

LITERATURE.—Hedin, Arch. f. ges. Phys., vol. xl, p. 360. Gärtner, Wien. klin. Woch., 1892, No. 2. Daland, Fort. d. Med., 1891, No. 21. Aspelin, Zeit. f. klin. Med., 1903, vol. xlix, p. 393. J. A. Capps, Journ. Med. Research, December, 1903. P. Wroth, Johns Hopkins Hospital Bull. February, 1907.

ESTIMATION OF HEMOGLOBIN.

Hemoglobinometers.—While it is usually possible to form a fairly clear idea of the degree of anemia by direct inspection of the patient, the appearance of the mucous surfaces, etc., it is often desirable to obtain more definite information, and, above all, a numerical expression of the extent of the anemia. This is especially important in the diagnosis of certain forms of anemia, in which the "color index" plays an important part—*i. e.*, the ratio between the percentage of hemoglobin and the percentage of the red corpuscles, as compared with the normal. To this end, special instruments have been devised, which are termed *hemoglobinometers* or *hemometers*. Of the various forms which are now in the market, the hemoglobinometer of Dare is probably the best, and is rapidly replacing the old instrument of v. Fleischl, which for many years was the standard. It is more exact and more convenient. Miescher's modification of the Fleischl instrument is possibly still more accurate, but too costly for general adoption. The little instrument of Gowers, in the modification of Sahli, when obtained from a reliable source will also furnish good results. Unfortunately many of those which have been placed on sale are worthless. Oliver's instrument has some advantages over the Fleischl, but none over the Dare. The Talquist method is warmly recommended by Cabot, and may be used to advantage in routine work by the general practitioner; for exact work it is insufficient.

Dare's Hemoglobinometer.—The essential parts of Dare's hemoglobinometer (Fig. 30) are an automatic pipette for collecting the blood (Fig. 31) and a graduated color scale (Fig. 32) to measure the corresponding percentage of hemoglobin. This latter reads from 10 to 120, the 100 mark corresponding to the color of a solution of 13.77 grams of hemoglobin in 100 c.c. of serum. The various shades of color corresponding to the scale are obtained by rotation of a prismatic glass semicircle tinted with golden purple of Cassius (Fig. 32, *E*), which is secured to a thin white glass disk (*I*). The numerical scale is placed on the edge of a corresponding semicircle (*H*) of thick white glass (*F*). This part of the apparatus is enclosed in a dust-proof hard-rubber case, and is rotated from the outside by the aid of a rubber-covered roller which runs on the edge of the disk and is turned by a milled wheel at *R* (Fig. 30). In the rubber case is a little circular window through which the color of the prism is viewed by means of a small telescoping camera tube (Fig. 33, *N*), provided with a magnifying lens of low power. The color aperture represents a surface about equal to 3 per cent. of the color scale. Looking through the tube a corresponding window will be seen side by side with the one through which the color scale is visible. In front of this the blood pipette is secured. The essential part of this is an oblong plate of white glass (Fig. 31, *A*), into the end of which a depressed surface of measured depth is ground, the floor being exactly parallel to the plane surface of the glass. This depression forms a capillary chamber (*D*) when the transparent glass plate (*B*)

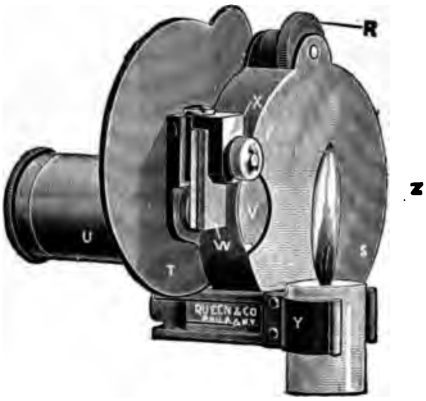

FIG. 30.—Dare's hemoglobinometer.

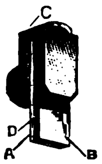

FIG. 31. Automatic pipette.

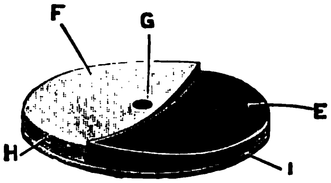

FIG. 32.—Graduated color scale.

is firmly clamped upon it by the pipette clamp *C;* it is filled by capillary attraction when either of the three free edges is touched to the blood drop. The pipette is held in position on the stage of the instrument by guides which run in grooves on the lower part of the clamp. The plate of white glass is toward the light.

The camera tube screws into a movable shutter (Fig. 30); when this is swung outward the two apertures become visible through which the blood and the colored scale are viewed.

In front of the pipette a candle is clamped in such a position that both the blood and the color scale are equally illuminated.

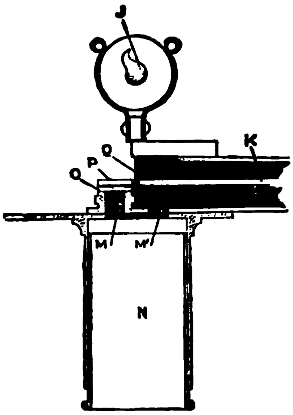

FIG. 33.—Horizontal section of Dare's hemoglobinometer. (on a level with centre of comparison apertures): *J*, candle; *K*, white glass disk of color prism; *L*, color prism; *M*, aperture through which color of blood film is viewed; *M'*, aperture through which the illuminated color prism is viewed; *N*, camera tube; *O*, transparent glass of pipette; *P*, white glass of pipette.

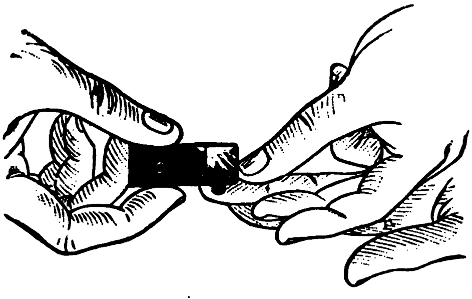

FIG. 34.—Filling the automatic blood pipette.

METHOD OF USE.—As the comparison of the color of the blood with that of the color scale should be made as soon after filling the pipette as possible, the apparatus is prepared for use beforehand by screwing the camera tube into place and adjusting the candle; this should be at such a level that the blue flame of the candle is below the color aperture, care being taken to have the wick of proper length (half-inch) and not charred at the tip. Curved or eccentric wicks should be turned so that the intensity of light in a vertical position is midway between the two color apertures.

The glass plates of the pipette having been thoroughly polished and refastened in the clamp, the finger or ear is freely punctured as usual and the capillary space of the pipette filled with the blood, by holding one of the three edges horizontally to the drop (Fig. 34). Any blood adhering to the flat surfaces of the glass plates is carefully wiped away and the pipette placed in position. The candle is lighted, the shutter thrown out, the camera tube focused, and the color of the blood (on the left) compared with the color scale (on the right). The two are matched by rotating the color disk by means of the milled wheel, which should be done in an abrupt manner, and frequently resting the eye. To this end the shutter is dropped and thrown out again as the case may be. The examination need not be conducted in a darkened room, but it is important to turn the instrument toward a dark background, so as to eliminate all direct or reflected light. The reading is indicated by the bevelled edge of the rectangular opening on the side of the case; the figure immediately beneath this represents the percentage of hemoglobin. Immediately after use, the two glass plates of the pipette are cleansed with water and a little acid alcohol, dried, and again replaced. Further details in regard to technique accompany the instrument.

My personal experience with the instrument has been quite satisfactory.

LITERATURE.—A. Dare, Phila. Med. Jour., Sept. 22, 1900.

Fleischl's Hemoglobinometer.—The principle underlying the v. Fleischl method is essentially the same as that of the Dare method; the color of the blood is compared with the color of a glass wedge stained with the golden purple of Cassius or a similar pigment, a scale indicating the corresponding amount of hemoglobin. With the Fleischl instrument, however, diluted blood is used, which is one of the disadvantages of the method.

The instrument (Fig. 35) consists of the glass wedge *a*, to which a scale, *b*, is attached, ranging from 0 to 120, 0 being placed at the thinnest, 120 at the thickest portion of the wedge. By means of a rack and pinion this may be made to slide from side to side beneath a platform corresponding to the stage of a microscope. In the centre of the platform there is a circular opening into which artificial light (daylight is not permissible) is projected from a circular plate of plaster of Paris mounted beneath, in the position of the mirror of the microscope. Into the circular opening a metallic tube, 1.5 cm. in height, is fixed, which is closed at the bottom with a plate of glass and divided into two equal compartments by a metal partition. One compartment receives the light through the glass wedge—the red chamber; the other, directly from the plaster-of-Paris reflector—the white chamber.

Capillary pipettes of known capacity accompany the instrument. This capacity is somewhat variable and is indicated on the handle of each, which number must correspond with that marked on the top screw head of the instrument. Generally speaking, the capacity of each pipette is such that with the blood of a perfectly normal individual the mixture of blood and water in the white chamber will correspond in color to that of the colored wedge at the mark 100 (a 13.77 per cent. solution of hemoglobin).

The pipette is filled by capillary attraction from a drop of blood obtained in the usual manner. If on trial it is found that the blood does not immediately run up in the tube, this is repeatedly washed out with water and then dried. If this is always done *after* the examination, the pipette will be in working order on the next occasion. While filling the pipette care should be had that it is not immersed in the blood, but only brought in contact with it. The two compartments of the cell having been previously partly filled with water, the charged pipette is at once placed in the white chamber and rapidly moved to and fro until the blood is well mixed with the water. Any trace remaining in the pipette is carefully washed out with water by the aid of a medicine dropper. The contents of the chamber are stirred with the handle of the pipette when both compartments are filled with water, using the same dropper, so that there is a convex meniscus over each. The color of the blood is then matched on the wedge, which should be moved by quick turns of the adjustment screw rather than in a gradual way, as the eye will otherwise be less apt to appreciate fine shades of difference. Daylight, as I have said, is not permissible; a candle or gas flame of moderate intensity placed about a foot and a half distant is best. The eye should be perpendicularly above the cell, and it is well to view the colors through a paper tube which is placed over the two compartments. The number facing the notch in the little well

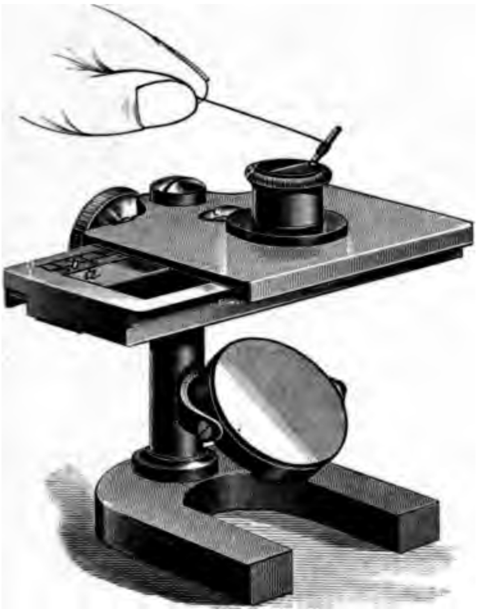

FIG. 35.—v. Fleischl's hemometer.

immediately behind the cell indicates the percentage of hemoglobin. The readings corresponding to the middle portion of the wedge are apt to be more nearly correct than the lower values. For this reason it is well, when a preliminary examination has shown a low figure, to repeat the test, using two or three pipettefuls of blood instead of one, the result, of course, being divided by 2 or 3, as the case may be. On the whole, the Fleischl method furnishes results which are somewhat lower than those obtained with the Dare; this is true especially of the older models, with which a percentage of 100 was only rarely observed. The instruments of more recent construction, however, are much better. Personally I regret to see the Fleischl apparatus supplanted by newer instruments; it was convenient and neat. It has its defects, to be sure, and it is unfortunate that the *Miescher modification*, in which these have been eliminated, and which unquestionably gives the most accurate results, is still so costly that its general use is out of the question.

Gowers' Hemoglobinometer (Sahli's Modification).—The apparatus (Fig. 36) consists of two glass tubes (*A* and *B*) which are of the same diameter. One of these (*A*) is closed and contains a solution of hematin hydrochlorate in a concentration corresponding to a 1 per cent. solution of normal blood. The other tube is provided with an ascending scale of 140 divisions, each degree corresponding to 20 cb. mm. A capillary pipette marked at 20 cb. mm., a guarded lancet, a dropping bottle, and a small stand accompany the instrument.

The finger is punctured as usual and the pipette filled to the 20 cb. mm. mark; the blood is immediately discharged into the graduated tube and mixed with one-tenth normal hydrochloric acid (saturated with chloroform as a preservative) which has been previously filled in to the mark 10. When the color of the mixture has become a clear dark brown, water is added drop by drop, shaking after every addition, until the color matches that of the standard solution. The division on the scale ultimately reached indicates the percentage of hemoglobin.

The examination can be conducted with natural and artificial light.

The method, as I have indicated above, is satisfactory if the instrument has been obtained from a reliable source. Its low cost makes it especially serviceable in large clinics and for purposes of teaching in the clinical laboratory. But in every case it is advisable to compare its scale with a standard instrument

Talquist's Method.—The color of the blood, in this case undiluted, is compared with a series of lithographed standard tints, which represent a scale ranging by tens from 10 to 100. The technique is very simple: drops of blood are received on pieces of white filter paper of suitable thickness which accompany the color scale, and are compared with the tints on the plate, using ordinary daylight.

Accuracy is, of course, not to be expected from so crude a method, so that its use is of necessity limited. It will suffice in a very general way to control the result of treatment, but it is inapplicable in the determination of the color index.

Estimation of Blood Iron with Jolles' Ferrometer.—The estimation of the hemoglobin from the amount of blood iron, as originally suggested by Jolles, is unfortunately not possible, as it has been shown that constant relations between the two bodies do not exist. All the iron of the blood is not present in this form, nor does it all occur in the form of colored compounds. Jolles' method of estimating the total amount of blood iron deserves consideration, however, as it is a practical method and discloses facts which are of clinical interest. It is desirable that it should be introduced in the clinical laboratory as a routine method.

The principle is the following: A small amount of blood is incinerated, and the remaining red oxide of iron brought into solution with a little monacid potassium sulphate. In this solution the iron is then estimated colorimetrically with an instrument which is constructed upon the principle of Fleischl's hemometer and which is termed the ferrometer. It is made by Reichert in Vienna and can be readily transformed into the hemometer proper. Full directions accompany the apparatus. The results are expressed in relative terms, the number 100 on the scale corresponding to 0.0425 per cent. by weight of iron. Some of the results which have been obtained with the clinical ferrometer are given below, together with the corresponding figures indicating the amount of hemoglobin:

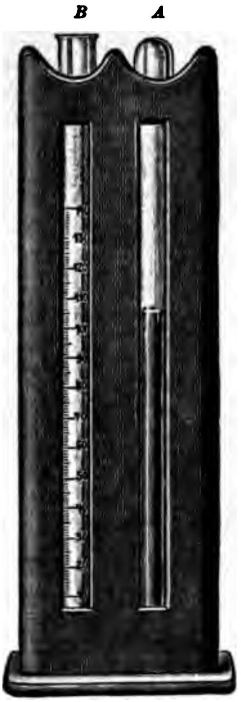

FIG. 36.—Sahli's hemoglobinometer.

	Ferrometer number.	Hemometer number.
Normal	103.0	100
Normal	92.6	105
Normal	95.5	100
Normal	110.0	105
Normal	83.8	92
Chlorosis	32.1-68.2	30-65
Simple anemia	33.2-74.7	15-40
Icterus	55.0	80
Leukemia	40.7	32
Leukemia	38.6	35
Pseudoleukemia	77.24	75-80
Severe diabetes	78.7	30
Severe diabetes	91.4	35-40
Parenchymatous nephritis	51 7	50

These figures at once illustrate the lack of relationship which exists between the amount of hemoglobin and that of the blood iron as a whole.

In a series of cases Jolles also examined into the presence of iron in the serum, by centrifugating a given volume of blood mixed with an 0.8 per cent. salt solution, and found that in health the serum contains no iron. In 3 cases of chlorosis, in 1 case of leukemia, in 1 of neoplasm, and 1 of interstitial nephritis, negative results were likewise reached. In 2 cases of severe diabetes, on the other hand, notable quantities were found.

Deganello[1] has studied the relation between the amount of blood iron and hemoglobin $\left(\frac{\mathrm{Fe}}{\mathrm{Hb}}\right)$ in different forms of secondary anemia, and found that this ratio remains normal, until the Hb has reached a certain minimum—46 to 58 per cent.; from this point off the value $\frac{\mathrm{Fe}}{\mathrm{Hb}}$ surpasses the normal the more the deeper the Hb value falls. Mere mechanical loss of Hb does not materially alter this value, however, even in cases of marked oligochromemia. When toxic influences are at play marked discrepancies will result.

Mitulescu[2] comes to quite analogous conclusions. He thinks that the hemoglobin estimation only is required as a rule, from which the iron value can be calculated according to Hoppe-Seyler's formula: $\mathrm{Fe} = \frac{\mathrm{Hb} \times 0.42}{100}$. If hemolytic processes are suspected, or if albuminuria exists, both methods are to be employed.

LITERATURE.—A. Jolles. "Ferrometer," Deutsch. med. Woch., 1897, No. 10; ibid., 1898, No. 7. Hladik, "Untersuchungen über d. Eisenghalt d. Blutes gesunder Menschen," Wien. klin. Woch., 1898, No. 4. S. Jellineck, "Ueber Färbekraft und Eisengehalt d. Blutes," ibid., Nos. 33, 34. A Jolles, "Vereinfachtes klin. Ferrometer," Berlin. klin. Woch., 1899, No. 44, p. 965.

KRYOSCOPIC EXAMINATION OF THE BLOOD.

The kryoscopic examination of the blood has for its object the determination of the molecular concentration, and hence of the osmotic pressure of the blood. The method is essentially based upon the observations of Raoult: (*a*) that all solid, liquid, or gaseous substances when dissolved in a liquid will lower the freezing point of that liquid; (*b*) that the degree to which the freezing point is lowered is dependent upon the amount of substance which is present in solution; and (c) that equimolecular solutions have like freezing points.[1] It follows that the freezing point of a solution furnishes an index of its molecular concentration, and hence also of its osmotic pressure, as this has been shown by van't Hoff to be proportionate to the number of molecules present.

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[1] Atti del R. Istituto Veneto di Scienze lett. and arti. T. lxiii.

[2] Zeit. f. klin. Med., lxx, 344, 190.

The degree to which the freezing point is lowered is designated by the letter Δ. In the case of normal blood this varies between —0.56 and —0.58° C., as compared with distilled water. A further depression is probably always indicative of renal insufficiency; it is a symptom of decided value and deserves more general consideration. In the domain of renal surgery especially the study of kryoscopy of the blood is important. Of foreign investigators, Kümmel more particularly has pointed out the value of the method in this field. As the result of 265 freezing-point determinations of the blood, in 170 cases in which various operations were performed upon the kidney and in which a direct examination of the organ was possible, he concludes that kryoscopy furnishes the most important index of renal insufficiency as compared with all other modern methods. Other observers, such as Casper and Richter, Tinker, and others, have arrived at similar conclusions. To Koranyi, however, belongs the credit for the introduction of kryoscopy into the clinical laboratory and its application to the study of renal diseases. Senator, Claude and Balthazar, Albarran, Kövesi, Lindemann, Waldvogel, and others have materially contributed to establish its value as a clinical method.

Zangemeister,[2] who has carefully studied the molecular concentration of the blood during pregnancy, the puerperal period, and in eclampsia, found a lessened concentration in the first instance, and values in the second which were still below the normal average and yet slightly higher than in pregnancy. In eclampsia the average concentration was quite normal. Similar results have been obtained by others, such as Füth and Krönig,[3] Szili,[4] and Lobenstine.[5] During pregnancy (ninth month) the latter found the average Δ in 12 cases to be —0.51° (variations from 0.45° to 0.57°); the average value in 12 puerperal women was —0.53° (variations: —0.49° to —0.58° C.). He accordingly concludes that if there is retention in eclampsia it must be of either colloidal substances or of crystalline substances, too small in amount to affect the concentration of the blood.

Schmidt[6] has recently studied the kryoscopic behavior of the blood in pneumonia and as the result of an analysis of 24 cases he concludes as follows:

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[1] Solutions are termed equimolecular when for a constant quantity of the solvent they contain such quantities of substance in solution that these bear the same ratio to each other as their molecular weights. Example: The molecular weight of sodium chloride is 58.5 and of sodium carbonate 106; if we dissolve these quantities or the same multiples of each in a constant quantity of water, such solutions would be equimolecular.

[2] Zeit. f. Geburts. u. Gyn., vol. i, Heft 3

[3] Centralbl. f. Gyn., 1901, No. 25.

[4] Orvosi Hetilap, 1900, No. 37.

[5] Amer. Med., October 15, 1904.

[6] Jour. Amer. Med. Assoc., Sept. 23, 1905.

There is an absolute lowering of the freezing point in pneumonia, which seems to depend either on the extent of the consolidation or on the height of the temperature or both. The concentration of the blood increases, as shown by the lower freezing point, as the disease progresses up to the time of the crisis. In those cases where the heart weakens perceptibly the freezing point of the blood becomes lower and in the fatal cases in which the heart gives out the freezing point is very low.

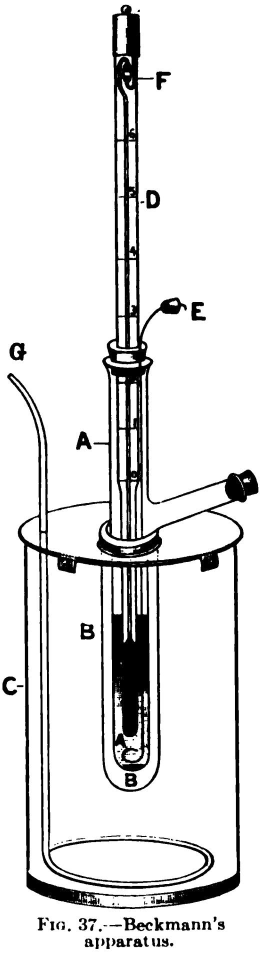

Fig. 37.—Beckmann's apparatus.

Method.—In the clinical laboratory a modification of Beckmann's apparatus is most conveniently employed (Fig. 37). Its essential parts are: a Heidenhain thermometer (*D*) graduated in hundredths and reading from —1° to —5° C.; a platinum wire loop for stirring (*E*); a test-tube (*A*) which is closed by a stopper through which the thermometer and stirring wire pass, and which in turn is placed in a second larger tube (*B*) so as to be surrounded by an air space. The jar (*C*) is filled with a freezing mixture of salt and ice, the temperature of which should lie between —2° and —5° C. Into this is placed the second tube *B*. The test-tube *A* is charged with 20 c.c. of blood (if only 10 c.c. are available, this amount may suffice), obtained by means of a large aspirating syringe from one of the veins near the bend of the elbow, as in the case of a bacteriological examination of the blood; the thermometer is introduced and the stirring wire adjusted. The tube is placed directly in the freezing mixture until the mercury leaves the reservoir bulb (*F*); this is done to save time. It is then adjusted in the second tube, as shown in the illustration, and the blood constantly stirred with the platinum wire. The temperature falls more or less rapidly below the freezing point before actual freezing takes place; as this occurs it suddenly rises again owing to liberation of heat, and then remains constant for some time. This point represents the true freezing point. Later, if the tube is allowed to remain in the freezing mixture, the temperature may fall to that of the latter. The difference between the freezing point of distilled water and that of the blood is Δ.

In every case it is necessary to determine the true zero for each instrument separately, as this often varies somewhat owing to unavoidable errors incident to its construction. To this end the tube *A* is charged with three to four times the amount of distilled water which is necessary for one examination. The greater portion of this is frozen; the liquid portion is thrown away; the frozen water is allowed to thaw and is again frozen in part, a portion being again thrown away; the remainder is sufficiently pure for the examination.

The freezing mixture is prepared by packing alternate layers of ice and salt into the jar around the tube *B*, which is held in position while the ice is packed. Ice and salt are finally thoroughly mixed by stirring with a heavy wire ring and rod (*G*). If several examinations are to be made, the water which separates out is poured off and replaced by an additional amount of salt and ice.

The method is quite expeditious, and if everything is previously prepared the examination does not occupy more than ten or fifteen minutes.

LITERATURE.—v. Koranyi, Zeit. f. klin. Med., 1897, vol. xxxiii, and 1898, vol. xxxiv. Lindemann, Deutsch. Arch. f. klin. Med., 1899, vol. lxv. Albarron, Annal. d. mal. génito-urin., 1899. Senator, Deutsch. med. Woch., 1900, vol. xxvi, p. 48. Claude and Balthazar, Presse méd., 1900, vol. xviii, p. 85. Casper and Richter, Funktionelle Nierendiagnostik, Berlin. u. Wien, 1901. Kümmel, Centralbl. f. Chir., 1902, vol. xxix, p. 121 of Beilage. Tinker, Johns Hopkins Hosp. Bull., 1903, vol. xiv, p. 162.

STUDY OF THE OSMOTIC RESISTANCE OF THE RED CELLS.

JANOWSKY'S METHOD.—The red cells are first counted as usual, using a 3 per cent. sodium chloride solution as diluent. Then a second count is made; this time with a hypotonic (0.4 per cent.) salt solution and a dilution of 1 to 200. Ten minutes should be allowed to elapse before mounting the drop. At the end of this time even normally a certain number of red cells lose their hemoglobin. This number is expressed in percentage terms, pro 1 cb. mm. of blood. The examination should always be made upon an empty stomach and in accurate work the barometric pressure and in cases of heart disease the height of the blood pressure should also be taken into consideration.

Under normal conditions the corpuscular stability is subject to definite individual variations, which lie within very narrow limits. It is increased by physical and mental labor, diminished by baths and diet free from meats.

Jakuschewsky[1] found normal values in diabetes (excepting in coma), pseudoleukemia, the primary stages of syphilis, chronic gastritis, atrophic hepatic cirrhosis, subacute parenchymatous nephritis, pyelonephritis, hysteria, and minor chorea. In aortic aneurism the stability is high, but quite analogous to what is found in normal old people with physiological sclerosis.

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[1] Russ. med. Rundsch, 1904, p. 345

Increased stability associated with an increase in the severity of the clinical symptoms and *vice versa* was noted in the following conditions: typhoid and typhus fever, recurrens, croupous pneumonia, acute and chronic malaria, influenza, acute rheumatism, advanced pulmonary tuberculosis, intestinal tuberculosis; chronic parenchymatous and interstitial nephritis (in association with uremic symptoms); anemia, chlorosis, leukemia, catarrhal jaundice; Charcot-Hanot's (biliary) cirrhosis; attacks of cholelithiasis with bile retention; acute gout; myocarditis (with beginning insufficiency); organic heart disease during lack of compensation; the final stage of carcinoma of the stomach.

Jakuschewsky thinks that the determination of the corpuscular stability may be of prognostic significance—an increase or retarded diminution *ceteris paribus* indicating an aggravation of the condition—and at times also of diagnostic value (carcinoma of the stomach).

BACTERIOLOGY AND PARASITOLOGY OF THE BLOOD.

General Technique.—In order to obtain results of value it is usually necessary to procure the blood for bacteriological examination directly from a bloodvessel. To this end the most prominent superficial vein near the bend of the forearm is chosen. An hour or two before puncture the entire region of the arm is thoroughly scrubbed with tincture of green soap, rinsed with warm sterile water, and finally washed with alcohol and with ether. A bichloride compress (1 to 500) is applied and left *in situ* until everything is ready for aspiration. It is then removed, and the area thoroughly rinsed and scrubbed with sterile water. An assistant compresses the large bloodvessels above

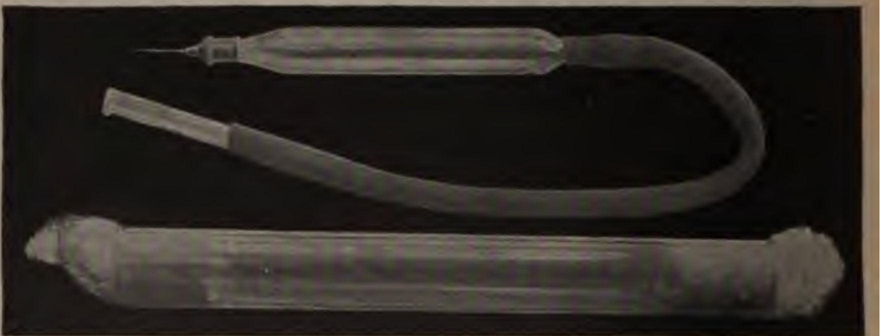

FIG. 38.—Blood aspirator; half size. (Ewing.)

the elbow with sufficient force to bring the superficial veins out prominently, but not to arrest the flow of blood. (A band firmly applied answers the same purpose.) For aspirating purposes the instrument pictured in the accompanying figure is more convenient than a hypodermic syringe. The tube is of about 10 c.c. capacity and graduated in c.c.; it is ground at one end so as to fit a No. 42 hypodermic needle. The glass tube contains a small plug of cotton at the far end. Needle and tube (minus rubber tube) are sterilized in a large test-tube by dry heat. When cool the rubber tube is slipped on. The needle is thrust obliquely into the most superficial vein (median basilic), being held almost parallel to the vessel. This is facilitated by steadying the bloodvessel with the fingers of the other hand (asepsis!). Blood flows immediately and this can be hastened by gentle aspiration. After the operation a small pledget of bichloride gauze is placed upon the site of the puncutre. As a rule the patients complain but little of pain, but in nervous persons a little ethyl chloride spray may be advantageously employed.

The contents of the tube are then at once divided among the culture media as described in detail below.

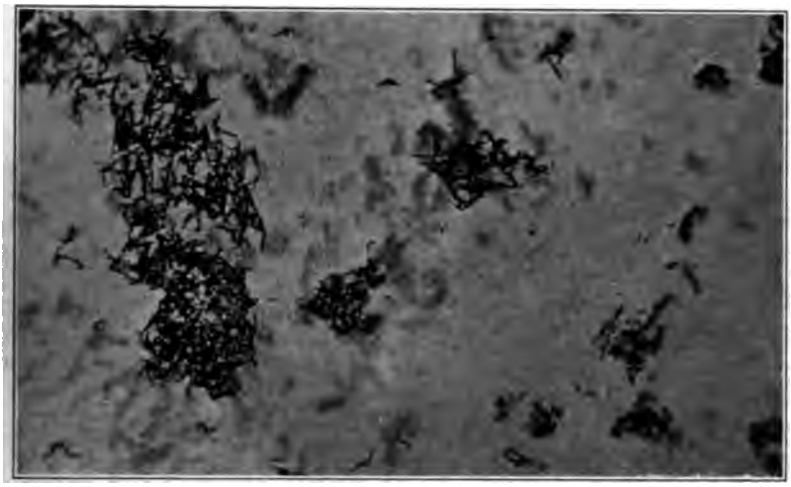

FIG. 39.—Positive agglutination reaction.

Typhoid Fever.—Recent research has shown that in typhoid fever the specific organism (Fig. 39) can be isolated from the blood in a large percentage of cases (over 80 per cent.) and at a time when the Widal reaction (see below) may not yet be demonstrable. In cases of great as well as moderate severity the organism is usually demonstrable during the entire febrile period, as also during relapses. It has hence been suggested that a bacteriological blood examination be made in all doubtful cases. The method is thoroughly practicable and deserves general recognition.

METHOD.—8 to 10 c.c. of blood are withdrawn from one of the superficial veins of the arm as described. Several Erlenmeyer flasks, each containing 150 c.c. of bouillon, should be ready at hand. Blood is added to these in varying proportions: two receive 1 c.c. each and two others 2 c.c. each. In this way 1 to 150 and 1 to 75 dilutions are obtained. The flasks are well shaken and placed in the incubator for twenty-four hours. A hanging drop is then examined.[1] If negative the incubation is continued for twenty-four hours further. When the bouillon has become cloudy, subcultures are made in milk and glucose bouillon (see description of typhoid bacillus) and the organism further tested with an actively agglutinating serum (see below).

It is interesting to note, however, that the tendency to agglutination of freshly isolated typhoid bacilli is almost invariably much inferior to that of bacilli which have been maintained for a long time on artificial media. Courmont thus notes that they were commonly agglutinated with a dilution of 1 to 50 by a serum which agglutinated laboratory bacilli at 1 to 200.

LITERATURE.—Neuhaus, Berlin. klin. Woch., 1886, Nos. 6 and 24. Schottmüller, Deutsch. med. Woch., 1900, No. 32. Castellani, cited in Presse méd., June, 1900. Auerbach u. Unger, Deutsch. med. Woch., 1900, No. 29. Cole, Johns Hopkins Hosp. Bull., 1901, p. 203. Courmont. Jour. d. physiol. et d. pathol. gén., 1902, vol. iv, p. 155. Polacco and Gemelli, Centralbl. f. inn. Med., 1902, vol. xxiii, p. 121.

Agglutination Test (Pfeiffer-Widal Reaction).—Owing to the development of specific agglutinins in the blood serum of typhoid patients, as a consequence of infection, such serum possesses the property of causing arrest of motility and agglutination of the corresponding bacilli. This observation, originally made by Pfeiffer, was utilized for diagnostic purposes by Widal, in 1896. The method which bears his name has been generally adopted in the clinical laboratory, and must be regarded as a most valuable aid in the diagnosis of typhoid fever. The reaction occurs in over 95 per cent. of undoubted cases, and may appear as early as the first day of the disease, meaning thereby the first day that the patient spends in bed or the fifth day of general malaise. Such instances, however, are uncommon, and, as a general rule, a positive result is obtained only after the fifth or sixth day in bed. In a small number of positive cases, on the other hand, the patient may pass through the entire course of the disease, and present typical clumping only during convalescence or a subsequent relapse. In every case, therefore, in which no reaction is obtained upon first trial, the test should be repeated at regular intervals throughout the disease.

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[1] At first the bacilli are but little active, but on further cultivation and reinoculation their motility increases.

Intermittence of the reaction, moreover, is very common, and emphasizes still further the necessity of frequent examinations in apparently negative cases.

While in some instances the reaction disappears very soon after the temperature reaches normal, and even earlier, it generally continues into convalescence, and may in some cases be observed months and years after the attack. Cases have been recorded in which a positive reaction could be obtained thirty-seven years after infection.

In a series of 71 cases who had had typhoid fever in the past Krause[1] found the reaction in 36—in 1 instance twelve years after the illness. Of 26 cases examined within a year 16 gave a positive result, of 21 from the second to the fifth year 12, of 19 from the fifth to the tenth year 7, and of 5 from the tenth to the twentieth year 1. In 3 instances no reaction could be obtained within a month of the disease.

Such observations, of course, entail the usefulness of the test to a certain extent. For this reason the demonstration of a negative reaction early in a case which is followed by a positive reaction is a particularly valuable symptom, the early negative result eliminating the possibility that the subsequent positive finding could be referable to an antecedent typhoid.

The question whether or not Widal's reaction is a specific reaction of the typhoid organism can be answered in the affirmative, notwithstanding the fact that at times cases of apparently true typhoid fever are seen in which no clumping is obtained, and that the reaction has been observed in cases which were apparently non-typhoid. Such exceptions are due in part to faulty technique, viz., to too low a degree of dilution of the serum, the use of old or impure cultures, too long a time limit of observation, single negative tests, etc. On the other hand, there can be no doubt that typhoid bacilli are at times present in the body without giving rise to symptoms of typhoid fever. In a case of cholelithiasis, reported by Cushing, typhoid bacilli were found in the gall-bladder, and distinct clumping was observed with a dilution of 1 to 30, although no history of typhoid fever could be obtained. Another interesting apparent exception to the rule that the Widal reaction is only obtained in cases of typhoid infection is reported by Grünbaum,[2] who notes that he obtained a positive reaction in cases of febrile jaundice. His observations have since been amply confirmed. The biliary components *pro se* have manifestly nothing to do with the production of the reaction, however, as is shown by the observation of Kämmerer[3] who obtained agglutination in only 3 jaundice cases out of 50 (from the most diverse hepatic diseases). In the positive cases no doubt infection had occurred by some organism of the paratyphoid group, standing nearer to the typhoid than the colon bacillus.

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[1] Zentralbl. f. Bact., 1904, vol. xxxvi, No. 1.

[2] Cited by Durham, Brit. Med. Jour., 1898, vol. ii, p. 600.

[3] Berlin. klin. Woch., No. 23, 1904.

There can further be no doubt that individuals exist who are naturally immune against typhoid fever, and that some of the positive results which have been obtained in healthy individuals who have never had typhoid fever may be explained in this manner.

So far as the non-occurrence of the reaction in cases of apparently true typhoid fever is concerned we now know that infections occur with organisms which are closely related to the typhoid bacillus (the paratyphoid group) and which clinically resemble typhoid fever, but which give no reaction with the typhoid bacillus, unless in low dilutions. (See Paratyphoid Fever.)

Widal's test is a most valuable aid in the diagnosis of typhoid fever, but cannot be relied upon to the exclusion of other symptoms.

Technique.—The method is based upon the fact that typhoid serum will cause arrest of motility and agglutination of the specific bacilli even when diluted, whereas clumping of the same organism is obtained only with sera from other diseases and healthy individuals when these are used in a more concentrated form. The time limit at which clumping occurs is likewise an important factor, as non-typhoid sera are at times met with in which, notwithstanding a certain degree of dilution, agglutination occurs, providing that the specimen is kept for a long time. Both factors—viz., the degree of dilution necessary to eliminate the agglutinating power of non-typhoid sera, as also the time limit of observation—have been arbitrarily determined. Widal originally advised a dilution of 1 to 10, and Grüber a time limit of one-half hour. It was soon ascertained, however, that this dilution was too low, and most observers have favored a dilution of 1 to 40 or 1 to 50. At the present time there is a tendency to further increase this even as far as 1 to 200 with a time limit of one-half hour.

With the original method only a full virulent, fresh bouillon culture of the typhoid bacillus, viz., one not older than sixteen to twenty-four hours, is employed. The further technique is simple: 1 volume of blood serum is diluted with the requisite amount of normal salt solution to 20, 25, 50, or 100 volumes, as the case may be. Of this mixture one droplet is mounted on a cover-glass, mixed with a droplet of the typhoid culture (dilutions of 40, 50, 100, or 200 thus resulting), and inverted over a cupped slide, with a little vaselin along the edges. The examination is conducted with a medium power (Leitz, 6 or 7; Bausch and Lomb, $\frac{1}{6}$).

If the case in question is one of typhoid fever, it will be observed that after a variable length of time the individual bacilli, which at first actively dart about the field of vision, become quiescent and tend to gather in distinct clumps, while the interspaces become entirely free from bacilli or very nearly so. After one-half hour, or one or two hours, according to the degree of dilution, all motion has ceased. When the time limit has expired and loss of motility and agglutination have not occurred the result is negative. In such an event further examinations should be made on the following days. In every case it is well to make a control test with the simple bouillon culture, so as to ensure the absence of preformed clumps and the virulence of the organism; of the latter, the degree of motility is the best index. In order to secure the necessary degree of dilution, various methods have been suggested. The simplest, and the one generally employed in municipal bacteriological laboratories, is to receive a large drop of blood upon a slide or slip of glazed paper, and allow it to dry. A drop or two of distilled water is then placed on the blood and allowed to remain for several minutes, when it is further diluted and examined as described. The principal advantage of this method is its simplicity and the fact that the *dried* blood retains its agglutinating properties for weeks and months. The results, however, are less reliable than with the use of liquid blood. This can be readily collected in little glass capsules, such as Wright has recommended for opsonic work (Fig. 40, *b*). The finger or ear is pricked as usual and the blood allowed to enter the bent capillary arm of the capsule by merely being held in contact. When enough has been collected, the far end of the capsule is warmed and the straight end sealed, when the blood will mount into the body of the capsule. The bent arm is then also sealed. In this manner the blood can be kept for a long time. At the laboratory it is hung into the centrifuge, if the serum has not already separated out, briefly centrifugated, and the capsule cut with a file. The serum is then diluted with the aid of a Thoma-Zeiss pipette or a common capillary pipette such as anyone can construct and is pictured in Fig. 40. These pipettes are destroyed after use.

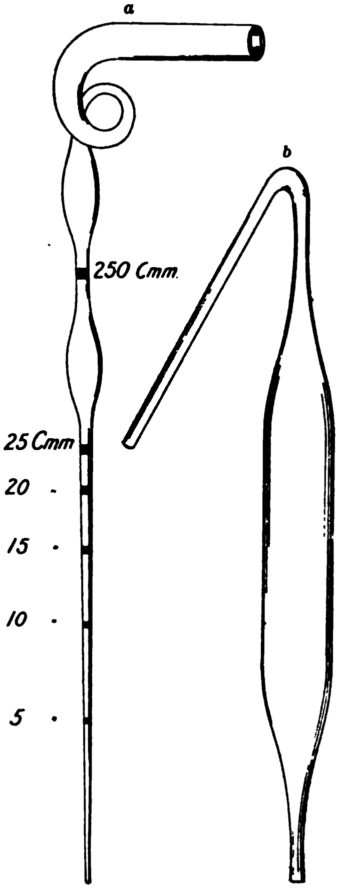

FIG. 40.—*a*, Wright's pipette; *b*, Wright's blood capsule.

A very material advance in the practical application of the agglutination test was made by the discovery that it is not necessary to work with living cultures of the typhoid bacillus, but that dead bacilli will answer just as well, providing they are killed off when in a virulent condition. To this end formalized cultures are especially convenient. To prepare this a twenty-four to forty-eight hours' bouillon culture of an actively agglutinable strain is treated with formalin to the extent of 1 per cent. of the solution, and set aside for a week. The bacteria are allowed to settle, when the supernatant fluid is poured off and replaced by formalized normal salt solution. In this form the material will keep for months. Before use it should be well agitated and examined to see that no artificial clumps are present. With the formalized culture the microscopic examination can then be made, or one can proceed macroscopically. If the microscopic test is used the examination is made after two to twenty hours.

The so-called *Ficker Diagnosticum* is a suspension of typhoid bacilli which have been killed off by a special process, which has not been made public. The outfit is sold by Merck and is used in the macroscopic application of the test. It consists of a series of small corked tubes, a graduated dropping tube, a bottle of the diagnosticum and one of normal salt solution, a small cupping glass and lancet. Cupping glass, rubber stopper, and lancet must first be sterilized by boiling in water. The blood is obtained from the back of the patient by making three or four deep punctures[1] and applying the cupping glass in the usual manner, viz., after placing a few drops of alcohol in the bottom and igniting it and rapidly placing the bottle to the skin before the flame is extinguished. The skin of the back is first cleansed with soap and water, alcohol, and ether. About 1 c.c. of blood is thus drawn, the bottle closed with the rubber cork and set aside in a cool place until the serum has separated. The test-tube and pipette are sterilized by means of alcohol and ether and the corks by boiling in water; 0.1 c.c. of the *clear* serum is now placed in one of the test-tubes, and after washing the pipette with water, alcohol, and ether, diluted with 0.9 c.c. of normal salt solution. A dilution of 1 to 10 thus results. The mixture is well shaken, and 0.1 c.c. placed in a second tube and 0.2 c.c. in a third. With the carefully washed pipette 0.9 c.c. of the diagnosticum is added to test-tube No. 2 and 0.8 c.c. to No. 3. Dilutions of 1 to 100 and 1to 50 thus result. A further tube (No. 4) receives 1 c.c. of the diagnosticum alone. All tubes are closed, well agitated, and set aside in the dark at room temperature. They are inspected after ten to twelve hours, when as a rule a positive reaction can be detected. Sometimes it is necessary to wait for twenty hours; if after that the result is negative it is so reported. If the reaction is positive the bacilli in tubes 2 and 3 will have fallen to the bottom, leaving the supernatant fluid clear, while the control tube 4 remains turbid. All tubes should be viewed against a dark background.

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[1] I find it more convenient to collect the necessary amount of blood from the ear; from 1 to 5 c.c. can be obtained by ordinary puncture without difficulty.

The results which are obtained with Ficker's diagnosticum are very satisfactory. The method has been amply investigated and uniformly endorsed.

The formalized cultures described above can be utilized just as well as the diagnosticum and in the same manner or any other modification that may suggest itself to the individual worker[1]. Capillary pipettes such as the one pictured in Fig. 40, *a*, can be used in the place of the special, calibrated instrument mentioned.

Paratyphoid Fever.—In cases of so-called paratyphoid fever organisms may be met with in the blood which apparently occupy a position intermediate between the typhoid bacillus and the organisms belonging to the colon group. Collectively they are spoken of as paratyphoid bacilli, though the question whether or not they represent well-defined species has not been definitely settled.

Cases of paratyphoid fever clinically resemble true typhoid, but are on the whole milder in their course. As a rule the serum does not react with the typhoid bacillus, while the organism which appears to be pathogenic in the individual case is agglutinated in a typical manner. Unfortunately, however, the serum of one case will not always react with the organism of a second case; so that the serum reaction will not always make it possible to distinguish the intermediates as a group from typhoid on the one hand, and the bacillus coli on the other.[2] Moreover, it has been shown that the serum of true typhoid may agglutinate the paratyphoid bacillus in higher dilutions even than the typhoid bacillus, although this is probably not usual (Grünberg and Rolly).

The paratyphoid group includes Widal's paracolon bacillus, Gwyn's bacillus, Cushing's bacillus 0, Hewlett's bacillus *b*, Noonan's bacillus, Schottmüller's bacilli, etc. It is subdivided into two groups, A and B, of which B causes at first an acid reaction in litmus milk, which in about ten days changes to a permanently alkaline reaction, while group A causes permanent acidity. Unlike the typhoid bacillus the paratyphoid ferments dextrose with the formation of gas. The intermediates do not form gas in lactose nor in saccharose media. On potato the growth is slight and there is no discoloration. They do not ferment milk or produce indol.

The examination of the blood is conducted as in typhoid fever, but it is not always necessary to dilute it to the same degree. Sometimes successful cultivation follows the spreading of a few c.c. of blood over the surface of agar tubes or plates.

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[1] Rüdiger, Jour. Infect. Dis., 1904, vol. i, p. 236.

[2] This only holds good for members of the paracolon group, while those of the paratyphoid groups interact without exception.

LITERATURE.—Gwyn, Johns Hopkins Hosp. Bull., 1898, vol. ix, p. 54. Cushing, ibid., 1900, vol. xi, p. 156. Schottmüller, Zeit. f. Hyg., 1901, vol. xxxviii. W. B. Johnston, Am. Jour. Med. Sci., 1902, vol. cxxiii, p. 187 (analysis of all cases reported up to that time). A. W. Hewlett, ibid., p. 200. Coleman-Buxton, ibid., 1903, vol. cxxiii, p. 979. See also Ascoli, Zeit. f. klin. Med., 1903, vol. xlviii, p. 419.

Pneumonia.—According to Rosenow the pneumococcus can be recovered in practically all cases of pneumonia, providing that large quantities of blood are used. He does not think that their presence indicates an especially unfavorable prognosis. He obtained positive results in 160 of 175 cases with a mortality of 40 per cent.

Positive results were obtained as early as twelve hours after the initial chill and as late as forty-eight hours after the crisis, although negative results are the rule after crisis. Pneumococci were also obtained in the blood smears in 47 out of 80 cases. The results of other modern investigators are similar.

Prochaska, working under Eichhorst's direction, found pneumococci in the blood in each of 10 cases examined, and in a subsequent series of 40 cases, of which 7 were fatal, he obtained the pneumococcus in 38.

Fränkel states that according to his experience, which is based upon an examination of more than 150 cases, one may infer that death will occur either with the symptoms of sepsis or that metastasis will take place in the internal organs whenever a larger number of colonies develop on spreading 1 c.c. of blood upon a plate of agar. If, however, the number is so small that it is necessary to take larger amounts of blood to demonstrate their presence and to grow them in bouillon instead of on agar, so as to eliminate the bactericidal power of the blood altogether, then Fränkel believes their presence is of no significance, and does not warrant a fatal prognosis. In the latter case he has found that the bacteria are frequently avirulent.

The examination, which should be repeated every day, if necessary, is conducted as follows: After disinfection of the arm in the usual manner 10 c.c. of blood are aspirated and agar tubes—liquefied at 40° C.—inoculated, each with 1 or more c.c. of the blood. Plates are then prepared and kept at a temperature of from 35° to 37° C. The colonies appear as small, round, grayish, jelly-like drops, which are quite characteristic.

Rosenow finds that the best results are obtained with blood agar. Upon this the pneumococci, especially when very virulent, produce a hemolytic zone which is greenish in color. This phenomenon, according to Schottmuüller, may serve to distinguish the pneumococcus from streptococci, which cause hemolysis without pigment production.

Instead of agar, bouillon may also be employed, and it is quite likely, as Prochaska suggests, that in this manner positive results may be more frequently obtained. Cole recommends the use of sterile litmus milk, of which portions of 150 c.c. each are employed in Erlenmeyer flasks. Early acidification and coagulation occur, and it is thus possible to determine more readily and quickly whether growth has taken place. Smears are then made and examined for capsules (see below). The identity is established by the characteristic shape and staining reactions of the organism, including the staining of the capsules, by the typical growth in milk and agar, and by the absence of growth, or very slight growth, in gelatin at ordinary room temperature. Especially characteristic, further, is the fermentation of inulin by the pneumococcus. To this end serum water containing inulin is used as recommended by Hiss.

The individual organism (Fig. 41) is capsulated, and usually occurs in pairs, arranged end to end or in short chains. At times, however, the chains are quite long, and then it may be difficult to distinguish it from streptococci. It is easily stained with the common aniline dyes. In order to differentiate the capsule, the method suggested by Epstein (see Sputum) should be employed. It should be remembered that capsules are only demonstrable in specimens obtained from milk or blood-serum cultures, while they are not shown in growths obtained from agar or bouillon.

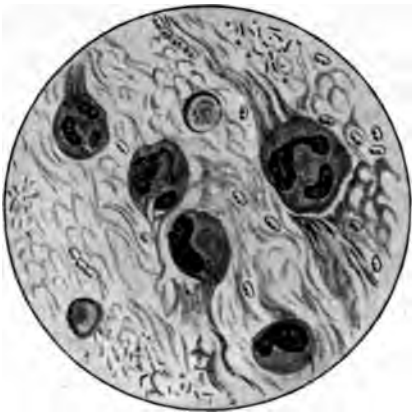

FIG. 41.—Pneumococcus, showing capsule.

Agglutination.—According to Rosenow pneumococcic serum invariably agglutinates the pneumococcus with a maximum dilution of 1 to 40 or 1 to 50. With rabbit-immune serum and using his serum-water medium for the growth of the organism, Hiss obtained agglutination with dilutions up to 800 and over.

LITERATURE.—Goldscheider, Deutsch. med. Woch., 1892, No. 14. Sittmann, Deutsch. Arch. f. klin. Med., 1894, vol. liii, p. 323. Kühnau, Zeit. f. Hyg., 1897, vol. xxv. Kohn, Deutsch. med. Woch., 1897, p. 136. James and Tuttle, N. Y. Presbyterian Hosp. Rep., vol. iii, p. 44. Sello, Zeit. f. klin. Med., 1898, vol. xxxvi. White, Jour. of Exper. Med., 1899, vol. ii. Silvestrini and Sertoli, Riforma Med., 1899, No. 116. Abstr. in Centralbl. f. inn. Med., 1899, vol. xxi.

R. Cole, Johns Hopkins Hosp. Bull., 1902, vol. xiii, p. 236. Prochaska, Centralbl. f. gen. Med. 1900, No. 46. Prochaska, Deutsch. Arch. f. klin. Med., vol. lxx, p. 559. Fränkel, Deutsch. med. Woch., 1901, V. B., p. 212. Rosenow, Jour. Amer. Med. Assoc., 1905, No. 2, p. 851; Jour. Infect. Dis., March, 1904.

Pyogenic Bacteriemia. Technique.—The general technique is the same as that described before, but large amounts of blood are advised, viz., 20 to 25 c.c. The media which are commonly employed are the ordinary laboratory media; in addition Libman has suggested the use of serum-glucose agar and serum-glucose bouillon. He has pointed out that on these media the growth of most bacteria is more marked and more rapid than on ordinary serum agar. This is true especially of the streptococcus, the pneumococcus, the gonococcus, and the meningococcus. With the solid media plates are employed almost altogether to the exclusion of media in tubes; 2 to 3 c.c. of blood are used for 15 to 20 c.c. of the solid media.

The number of organisms which may be found in the blood in septic conditions is exceedingly variable. On the one hand, but one plate or flask out of several may show any growth, and then only after several days; while, on the other hand, the number of organisms may be quite large. Cole has reported a case of streptococcus septicemia in which the number of organisms amounted to 3642 per cubic centimeter of blood six days before death, and then rose to 10,716 per cubic centimeter two days before death. I have seen a case of meningococcus septicemia in which the organisms numbered 7,380,000 per cubic centimeter just before death.

The time before death at which organisms may be found in the blood is also quite variable; sometimes they may be demonstrable a month before, in other cases only a day or two before the fatal issue.

(*a*) **Staphylococcus Bacteriemia.**—Staphylococcus bacteriemia is more common than was formerly supposed. The variety usually seen is the Staphylococcus aureus. The albus is rare. Libman states that the latter plays an insignificant role in systemic infections; that in several years he has not met with a single instance in which he could ascribe a systemic infection to the Staphylococcus albus. He draws attention to the fact that the pigment production in the aureus may be delayed and that some of the positive albus cases recorded in the literature may in reality have been aureus cases of this kind. He accordingly recommends that an apparent albus be observed for five days and grown upon potato and serum agar before the diagnosis is made (glucose interferes with pigment production). Staphylococcus citreus also is very rare.

Of the 28 positive findings in Libman's large series of blood cultures many were instances of osteomyelitis, some were secondary to furuncles or cellulitis, others were cryptogenetic, and 2 referable to post-partum infection (rare). All these were aureus cases. The only positive albus cases were obtained within forty-eight hours before death; Libman looks upon these as agonal invasions. The Staphylococcus citreus was isolated once in a case of osteomyelitis.

F. Meyer and Michaelis and others report having found pus organisms in a large percentage of cases of advanced phthisis. This is denied by Jochmann, excepting as agonal infections.

The *Staphylococcus pyogenes aureus* occurs in the form of spherical bodies, averaging about 0.8 μ in diameter, which readily stain with the basic aniline dyes, as also with Gram's method. They usually occur in clumps, but may also be seen in pairs and in short chains. The organism grows on all culture media, and in the presence of oxygen gives rise to the formation of an orange-yellow pigment. Gelatin is rapidly liquefied; it coagulates milk with acid reaction and clouds bouillon. The *Staphylococcus pyogenes albus* and *citreus* differ from the aureus by the absence of pigment in the first and by the formation of a lemon-yellow pigment in the second.

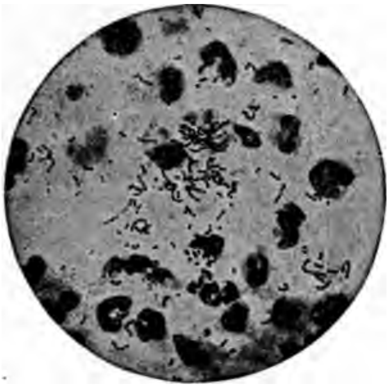

FIG. 42.—Streptococcus pyogenes. × 800. (Fränkel.)

(*b*) **Streptococcus Bacteriemia.**—In the large series of blood cultures reported by Libman streptococci were isolated in 58 cases. Streptococcus bacteriemia is thus more common than staphylococcus bacteriemia. Some were instances of terminal infections, or infections arising from the tonsils, the ears, and mastoid processes, or the genitourinary tract (abortion and postpartum infections), while in others infections were referable to wounds, and still others were cryptogenetic. Some cases were characterized by joint or bone lesions. Endocarditis was frequent. One was a case of erythema nodosum. In several cases of mild acute endocarditis, following what clinically appeared to be typical articular rheumatism, Libman found attenuated streptococci. They could be demonstrated during extended periods of time.

Streptococci have also been found in the blood in advanced cases of phthisis (probably as agonal invasions).

Hektoen has pointed out that in scarlatina streptococci may be found in the blood during life in at least 18 per cent. of all cases.

I append his conclusions: Streptococci may occasionally be found in the blood of scarlet-fever cases that run a short, mild, and uncomplicated clinical course. They occur with relatively greater frequency in the more severe and protracted cases of the disease, in which there may also develop local complications and clinical signs of general infection, such as joint inflammations; but even in the grave cases of this kind spontaneous recovery may take place. In fatal cases streptococci may not be demonstrable. The theory that scarlet fever is a streptococcus disease thus does not seem to receive direct support from these investigations.

In diphtheria, measles, and smallpox infection with streptococci is also not uncommon. Other organisms may, however, also be met with, such as the various staphylococci, and quite commonly also, according to Jehle, the bacillus of influenza.

The *Streptococcus pyogenes* (Fig. 42) occurs in chains of spherical cocci which usually vary from four to twenty in number. The size of the individual organism is somewhat greater than that of the staphylococcus, but may vary even in one and the same chain. It is readily stained with the basic aniline dyes and also with Gram's method. It grows on all culture media at the temperature of the room, forming small, gray, granular colonies on agar and gelatin. Unlike the pneumococcus it does not ferment inulin media. As a rule, it does not liquefy gelatin, and it may or may not coagulate milk and cloud bouillon. Several varieties are recognized, viz., *Streptococcus brevis*, which forms short chains; *Streptococcus longus*, which occurs in long chains; streptococci, which render bouillon cloudy, and those which do not; streptococci, which form flocculent, sandy, scaly, or viscous sediments.

The *Streptococcus conglomeratus* grows, without clouding bouillon, in the form of dense, separate particles, scales, or thin mebmranes at the bottom and sides of the tube, and on shaking the sediment it breaks up into little specks, without producing uniform, diffuse cloudiness. The chains are long and interwoven in conglomerate masses (Welch).

(*c*) **Non-pneumonic Pneumococcus Bacteriemia.**—In Libman's series, apart from the pneumonia cases, pneumococci were found only four times. Twice there was an acute endocarditis of unknown source, once there was an infection between two toes, and once there was a suppurating ethmoiditis and frontal sinusitis, with abscess. Other observers have found the organism in cases of biliary abscess at the time of the chill, in suppurative oöphoritis, in peritonitis, etc. It is interesting to note in this connection that Libman obtained only negative results in 25 cases of peritonitis, and also in a series of 25 cases of appendicitis, some of which were very severe.

(*d*) **Bacterium Proteus Bacteriemia.**—Libman reports a case of uremia in which the proteus was found one day before death, together with streptococci.

(*e*) **Colon Bacillus Bacteriemia.**—The colon bacillus also is rarely found in the blood. Libman mentions a case in which it was demonstrated where the operation of internal urethrotomy had been performed. An interesting case is also reported by Rochester (see literature below).

(*f*) **Paracolon Bacteriemia.**—Aside from those cases in which paracolon bacilli have been found in so-called paratyphoid fever, paracolon bacteriemia is very rare. Libman and Berg report one case which clinically resembled cholecystitis.

(*g*) **Bacillus Pyocyaneus Bacteriemia.**—The Bacillus pyocyaneus is rarely found in the blood. Libman and Brill report a case in which it occurred secondarily to a Staphylococcus aureus bacteriemia.

(*h*) **Gonococcus Bacteriemia.**—Cases of gonorrheal septicemia in which the gonococcus was isolated from the blood of the patients during life have been reported by Thayer-Blumer, Thayer-Lazear, Byelogoway, Wilson, Harris-Johnston, and others. In all these cases gonorrheal endocarditis existed. In other infections of the same nature positive results were obtained by Ahmann, Colombini, Panichi, and Unger, in association with polyarthritis, epididymitis, myositis, tendovaginitis, inguinal bubo, and parotitis. In the endocarditis cases cultures were obtained after an illness lasting for from five weeks to seven months, at times as early as the ninth to the eleventh day preceding death, and on an average five days before death.

To cultivate the gonococcus from the blood during life, it is neither necessary to use a large amount of blood nor to dilute it greatly, nor to employ any specially prepared medium. From 2 to 5 c.c. are sufficient. According to Harris and Johnston, it is more advantageous to mix the blood with melted agar and to plate the same than to use fluid media where the oxygen supply is more restricted. (For a description of the organism, see Gonorrheal Pus.)

LITERATURE.—N. M. Harris and W. B. Johnston, "Gonorrhœal Endocarditis with Cultivation of the Specific Organism from the Blood during Life," Johns Hopkins Hosp. Bull., 1902, vol. xiii, p. 236 (literature). Thayer and Blumer, ibid., 1896, vol. vi, p. 59. Thayer and Lazear, Jour. Exper. Med., vol. iv, p. 81.

(*i*) **Micrococcus Zymogenes Bacteriemia.**—This organism is apparently closely related to the Pneumococcus and the Streptococcus pyogenes. It has been isolated from the blood in one instance by MacCallum and Hastings.

(*k*) **Meningococcus Bacteriemia.**—In several instances of meningococcus meningitis the corresponding organism has been isolated from the blood. In one case I found 7,380,000 diplococci per cubic centimeter. The organisms could be demonstrated in large numbers directly in the blood smear. Almost all were enclosed in polynuclear neutrophiles and in large mononuclear elements.

Endocarditis.—In acute endocarditis or in acute exacerbations of chronic cases bacteriemia is fairly common. Lenhartz obtained positive results *intra vitam* in 16 cases out of 28, and Libman states that in cases of acute ulcerative endocarditis he has always found organisms in the blood. The organisms which have been encountered are the Staphylococcus aureus, streptococci, pneumococci and the gonococcus. Of these the streptococcus cases are the most common, while the staphylococcus comes next in order. Pneumococcus and gonococcus endocarditis is relatively uncommon. Libman remarks that there is often a marked disproportion between the number of bacteria in the blood and the extent of the lesion. There may be an almost countless number in the blood and only very small deposits on the valves, or there may be large vegetations with hardly any bacteria in the blood. As a rule they are present in fair numbers.

In a series of 10 cases of mild acute endocarditis following what clinically appeared to be typical articular rheumatism Libman could demonstrate attenuated streptococci and diplococci during extended periods of time.

Prognosis in Pyogenic Bacteriemia.—The prognosis in the pyogenic bacteriemias, aside from other considerations, is upon the whole unfavorable; recoveries, however, are possible. Each individual case must be judged separately. In Libman's series of 50 cases of streptococcemia there were 6 recoveries (11 per cent.); of 28 cases of staphylococcemia 8 recovered (nearly 29 per cent.); of his 4 pneumococcus cases 1 recovered. Leaving out the few pneumococcus cases there would be 86 cases with 16 per cent. recoveries.

In Bertelsmann's series of 48 cases of surgical bacteriemia 21 recovered, viz., 43 per cent.; of these there were 28 streptococcus cases with 19 recoveries and 13 staphylococcus cases with 4 recoveries.

In Lenhartz's series of 77 medical cases (including several post-partum infections), there were 17 recoveries; of these there were 47 streptococcus cases with 6 recoveries and 13 staphylococcus cases with 1 recovery.

LITERATURE.—F. W. White, "Cultures from the Blood in Septicemia, Pneumonia, Meningitis, and Chronic Diseases," Jour. Exper. Med., vol. iv, p. 425. Petruschky, Zeit. f. Hyg., vol. xvii, p. 59. Sittmann, Deutsch. Arch. f. klin. Med., vol. liii, p. 323. Canon, Deutsch. Zeit. f. Chir., vol. xxxiii, p. 571; and Mitth. aus d. Grenzgeb. d. Med. u. Chir., 1902, vol. x, p. 41. Lenhartz, Münch. med. Woch., 1901, Nos. 28 and 29. Libman, Proc. N. Y. Path. Soc., 1903, vol. iii, pp. 5 and 57; "On Certain Features of the Growth of Bacteria," etc., Jour Med. Research, 1901, vol. vi. Cole, Johns Hopkins Hosp. Bull., 1902, vol. xiii, p. 252. Wm. Welch, "Morbid Conditions Caused by the Bacillus Aërogenes Capsulatus," ibid., 1899, vol. x, p. 134. Gwyn, ibid., 1900, vol. xi, p. 185 (first case); Cole, ibid., 1902, vol. xiii, p. 234 (second case). Hektoen, Jour. Amer. Med. Assoc., 1903, vol. xl, No. 11. Jehle, Zeit. f. Heilk., 1901, vol. xxii, p. 190. Ewing, Trans. Amer. Assoc. Phys., 1902, vol. xvii, p. 208. D. Rochester, Jour. Amer. Med. Assoc., March 2, 1907. C. E. Simon, Meningococcus Septicæmia, Johns Hopkins Hosp. Bull., 1907.

Anthrax.—The bacillus of anthrax, as first pointed out by Pollender, Brouell, and Davaine, is frequently met with in the blood in the corresponding disease. As a rule the number is small. Smears are stained for five to ten minutes in a mixture of 30 c.c. of a concentrated alcoholic solution of methylene blue and 100 c.c. of a 1 to 10,000 solution of potassium hydrate; they are then washed for five to ten seconds in 0.5 per cent. acetic acid, washed with alcohol and dried. Thus stained, the bacilli appear as rods measuring from 5 to 12 μ in length by 1 μ in breadth, and usually present a segmented appearance, the extremities being slightly thickened. Under suitable conditions spore formation takes place. When present in large numbers it is not necessary to stain the blood, as the organism can then be seen without difficulty in the wet specimen.

In doubtful cases, in which a microscopic examination of the blood yields negative results, a few cubic centimeters may be injected into a mouse or a guinea-pig, in the blood of which the bacilli will soon be found in enormous numbers if the disease is anthrax.

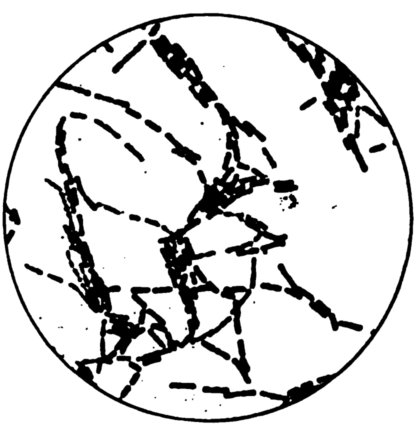

FIG. 43.—Anthrax bacillus. × 900 diameters. Agar culture. (Park.)

McFadyean has described a color reaction of anthrax blood which seems to be pathognomonic of the disease. Smears are prepared as usual and, when air-dry, fixed by heat—until the slide has become a little too hot to be held against the skin. On cooling the specimens are stained for a few seconds with a 1 per cent. aqueous solution of methylene blue (medicinal of Merck), or with one of Grübler's methylene blues, modified by boiling with ½ per cent. of sodium bicarbonate. After washing with distilled water they are dried with filter paper, subsequently by heat, and mounted in balsam. Anthrax blood then shows a distinct reddish or purplish tone, especially when held against the light, while other blood appears pure blue or greenish blue.

Microscopic examination of the amorphous intercellular material shows the same result.

According to Heim, who has described the same reaction independently of McFadyean, the color change is due to mucin derived from the capsules of the bacteria.

LITERATURE.—Pollender, Casper's Vierteljahrsch. f. gerichtl. u. öffentl. Med., 1855, vol. viii, p. 103. Brauell, Virchow's Archiv, vol. xi, p. 132, and vol. xiv, p. 32. Davaine, Compt.-rend. de l'Acad. d. Sci., vol. lvii, p. 220. Blumer and Young, Johns Hopkins Hosp. Bull., 1885, p. 127. McFaydean, Jour. Comp. Pathol. and Therap., March and December, 1903. Heim, Münch. med. Woch., 1904, No. 10.

Tuberculosis.—In acute tuberculosis tubercle bacilli have repeatedly been observed in the blood; but the search for them is most tedious and often in vain. Nevertheless a careful examination of the blood is indicated in doubtful cases; but only a positive result is of value.

According to Liebmann, the tubercle bacilli are most numerous in the blood about twenty-four hours after the injection of tuberculin. Working in this manner he claims to have obtained positive results in 56 cases of 141. As a rule it is best to resort to the animal experiment.

For methods of staining and a description of the tubercle bacillus, the reader is referred to the chapter on Sputum.

LITERATURE.—Liebmann, Berlin. klin. Woch., 1891, p. 393. Krönig, Deutsch. med. Woch., 1894, vol. v, p. 42.

Leprosy.—In leprosy the corresponding bacilli have been found in the blood by Mitsuda.[1] Their demonstration, however, is difficult.

Glanders.—In glanders the specific bacillus is constantly present in the blood, and may be demonstrated by staining dried preparations for five minutes with a concentrated alcoholic solution of methylene blue mixed with an equal volume of a 1 to 10,000 solution of potassium hydrate just before using. From this mixture the specimen is passed for a second or two into a 1 per cent. solution of acetic acid which has been tinged a faint yellow by the addition of a little tropeolin 00 solution; it is then decolorized by washing in water containing 2 drops of concentrated sulphuric acid and 1 drop of a 5 per cent. solution of oxalic acid for each 10 c.c. In specimens thus stained the bacilli appear as short rods measuring from 2μ to 3μ in length by 0.3μ to 0.4μ in breadth, often containing a spore at one end.

LITERATURE.—Duval, Arch. de méd expér., 1896, p. 361.

Influenza.—The influenza bacillus has been found in the blood occasionally, but is more readily demonstrated in the sputum. Jehle found it in 22 cases of scarlatina out of 48 that ended fatally, in measles 15 times out of 23, and in 5 cases of varicalla out of 9. In Hektoen's series, on the other hand, the organism was not found; but it is noted that during the year influenza was not especially prevalent in Chicago. (For a description of the organism see the Sputum.)

LITERATURE.—Canon, Virchow's Archiv, vol. cxxxi, p. 401. Klein, Baumgarten's Jahresb., 1893, p. 206. Kühnau, Zeit. f. Hyg., vol. xxv, p. 492.

Malta Fever.—In Mediterranean or Malta fever the specific organism, the *Micrococcus melitensis* (Bruce), has been isolated from the blood during life. It is said to be present in the peripheral blood in all cases during the early stages and in severe febrile relapses.

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[1] Folia hæmatol., vol. i, p. 502.

In the afebrile intervals and the subsequent cachexial stage it is not demonstrable. In no case are the organisms abundant, and for this reason the bacteriological findings are rather uncertain.

Diagnosis is facilitated by the fact that a well-pronounced agglutination is obtained with the patient's serum. A positive reaction with a dilution of more than 1 to 20, according to Birt and Lamb, may be regarded as proof positive of the existence of the disease. As a rule agglutination can still be obtained with a dilution of from 1 to 600 to 1 to 700. It begins about the fifth day of the disease, and gradually diminishes in intensity during convalescence, but may persist for a year and a half and even longer.

The organism in question is a coccus, measuring 0.3 μ in diameter, and occurs singly, in pairs, and sometimes in fours. Longer chains are not seen. It is motile. It is stained by the usual dyes and grows on nutrient agar and in broth. The colonies are usually not visible until the third day. At first their color is that of a transparent amber, while later they are opaque. Liquefaction does not occur.

LITERATURE.—C. Birt and G. Lamb, "Mediterranean Fever," Lancet, Sept. 9, 1899. Wright and Smith, Brit. Med. Jour., April 10, 1907. Musser and Sailer, Phila. Med. Jour., 1898, p. 1408, and 1899, p. 89. R. P. Strong and W. E. Musgrave. "The Occurrence of Malta Fever in Manila," Phila. Med. Jour., 1900, p. 996. J. J. Curry, "Malta Fever," Jour. Med. Research, July, 1901.

Bubonic Plague.—In advanced cases of bubonic septicemia the specific organism may be found in the blood in small numbers. Toward the end of rapidly fatal cases they become more numerous, and may then be demonstrable directly with the microscope. According to Bell[1] the bacilli can be found in all cases and at all stages of the disease by using Ross' dehemoglobinizing method (p. 177).

The organism in question, the *Bacillus pestis* (Kitasato, Yersin), is a short, thick coccobacillus, with rounded ends, measuring 1.5 μ to 1.75 μ in length by 0.5 μ to 0.7 μ in breadth. Examined in the hanging drop it is devoid of automobility. The polar regions are readily stained, while the interpolar area remains colorless. In many organisms a capsule can be made out by appropriate methods, but it is apparently not a constant feature. Oftentimes the form of the organism deviates from the normal. It may thus resemble a coccus on the one hand, while on the other it appears more elongated, and again it is common to meet with distorted and swollen, vacuolated forms, which are interpreted as involution or degeneration forms. These latter are especially numerous in older cases and old cultures. The organism is decolorized by Gram (Fig. 44).

The blood smears are fixed by immersion in absolute alcohol for twenty-five minutes; or they are covered with absolute alcohol for about one-half minute, when the alcohol is burned off. For staining purposes, borax methylene blue (a solution of 2 per cent. methylene blue in 5 per cent. borax-water) or Löffler's alkaline methylene blue may be conveniently employed. In the first instance we stain for one-half minute, in the second for two to three minutes. The polar staining is in this manner quite satisfactory.

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[1] Brit. Med. Jour. March 5, 1904.

On gelatin and agar containing 2.5 to 3.5 per cent. of salt and in bouillon a fairly characteristic growth results. In the case of the agar involution forms are obtained, among which long, slender bacilli, which are segmented and present a vacuolated appearance, are especially noteworthy. In this state they stain quite badly and have lost a certain degree of their virulence. In bouillon the organism often forms long chains of well-rounded bodies which are quite similar to a coccus. During its growth in bouillon it forms flakes or flocculi, which rapidly sink to the bottom of the tube, leaving the liquid clear above. Stalactite or stalagmite formations may also be seen, starting from the walls of the tubes or from suspended droplets of oil or butter. Colonies on gelatin about thirty-six hours old are warty, strongly refractive formations, which often present a delicate, irregularly indented margin. Even after twenty-four hours one can obtain smears in which 50 to 100 bacilli are grouped in little colonies of irregular form, while examination of the plates with a magnifying power of 60 diameters reveals scarcely any growth. The organism does not liquefy gelatin. The optimum temperature for growths is between 25° and 30° C.

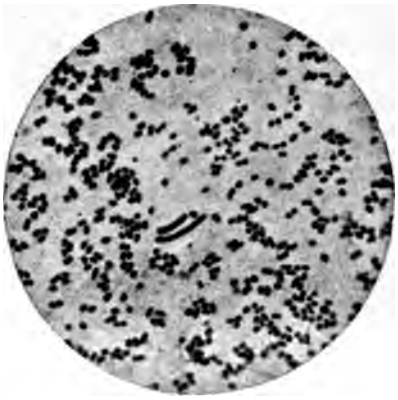

FIG. 44.— Plague bacilli from agar culture. × 1100 diameters. (Park.)

LITERATURE.—For Kitasato's report see Annual Rep. of the U. S. Marine-Hospital Service for 1894; W. Wyman, Bubonic Plague; U. S. Treasury Dept., 1900. Kossel u. Overbeck, Arb. aus. d. Kais. Gesundheitsamt., 1901, vol. xviii.

ANIMAL PARASITES.

Malaria.—Malarial fever is referable to infection with a specific protozoan parasite belonging to the class of hematozoa, representatives of which are found in the blood of various animals, such as the rat, frog, turtle, carp, various birds, etc. Three varieties are known to occur in the blood of man, viz., the parasite of tertian, quartan, and estivo-autumnal fever. The life history of these organisms is now well understood, and it is known that in addition to the intracorporeal cycle of development which takes place in the human body there is yet another, an extracorporeal cycle, which occurs in certain mosquitoes of the genus Anopheles. Infection occurs through the bites of such mosquitoes, which themselves have been infected by sucking the blood of malarial patients. This has been abundantly demonstrated by Ross, Manson, Grassi, and others, and may be regarded as an established fact.

Method of Examination.—When the patient is directly available at the laboratory, or if a few hours only need elapse before the examination is made, wet mounts may be used, which are best ringed with a little vaselin or paraffin, if they cannot be examined at once. Otherwise dry mounts are prepared and stained with the eosinate of methylene blue, or one of the Romanowsky dyes, such as Hastings', Wright's, Giemsa's, etc. (See Plate X.) With the Romanowsky mixtures, which all contain methylene azure, the chromatin (nuclear) granules are shown.

It is best to procure specimens shortly before an attack, as adult forms are then obtained; immediately after an attack is not the proper time to hunt for parasites.

In cases in which but few organisms are expected Ross has suggested the advisability of spreading thick blood specimens and extracting the hemoglobin before staining. The search for the youngest forms of the estivo-autumnal parasite especially is much facilitated in this manner. Ruge endorses this method in the following modification, but points out that the specimens are by no means beautiful. A large drop of blood (about 20 cb. mm.) is spread over a surface measuring about 18 square millimeters. The air-dried preparation is then placed for a few minutes in a 5 per cent. solution of formalin,[1] to which 0.5 to 1 per cent. of acetic acid has been added. In this manner the hemoglobin is all extracted, while at the same time the blood film is fixed; so that it can now be washed without fear of ruining the preparation. This is then stained either according to one of the modifications of the Romanowsky method or with the eosinate of methylene blue. Ruge further advises that specimens stained according to the Romanowsky method be subsequently stained with Manson's solution,[1] in order to render the smallest and medium-sized ring forms more readily visible, as their affinity for the dye is somewhat impaired by the fixation in formalin. My own experience with this method has been very satisfactory.

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[1] This solution would contain 2 per cent. of formaldehyde gas, as the commercial formalin is about a 40 per cent. solution.

Plasencia suggests the following method: Fixation in 0.5 per cent. formalin and absolute alcohol (equal parts); *rapid* drying in the air and washing in distilled water. The specimens are then stained with a mixture composed of 80 c.c. of a saturated aqueous solution of toluidin blue and 60 c.c. of a 1 per cent. aqueous solution of eosin. After washing in water they are dried and examined as usual. Plasenica regards this stain as better than Manson's.

The Parasite.—The following forms of the parasite may be found in the blood:

1. HYALINE NON-PIGMENTED INTRACELLULAR BODIES.—These apparently represent the earliest stage in the development of the parasite, and are found in all forms of malarial fever; they are especially abundant during the latter part of the paroxysm or immediately thereafter. At first sight they may be mistaken for vacuoles, but upon closer examination it will be found that they exhibit distinct movements of an ameboid character, and may thus easily be recognized with a little experience.

The rapidity with which these changes in form occur in the tertian type of ague is most astonishing, and sketches of any one phase can often, indeed, be made only from memory; in quartan fever the movements are much slower and far less extensive.

In the irregular fever of the estivo-autumnal form ameboid movements may likewise be observed, but more commonly the parasite assumes a ring-like appearance, and does not throw out distinct pseudopodia. If these forms are carefully observed, however, it will be found that they are not absolutely quiescent, but alternately expand and contract.

In tertian fever the organism (Plate VIII) is pale and indistinct, while in quartan fever it is sharply outlined and somewhat refractive (Plate IX, Fig. 2). In the estivo-autumnal form the organism is usually much smaller than in the tertian type, and the ring-like bodies frequently present at some point in their interior a distinctly shaded aspect which closely resembles the darker portion in the centre of a normal corpuscle (Plate IX, Fig. 1). It is thus possible, even at this stage in the development of the parasite, to distinguish between fever of the tertian, quartan, and estivo-autumnal type.

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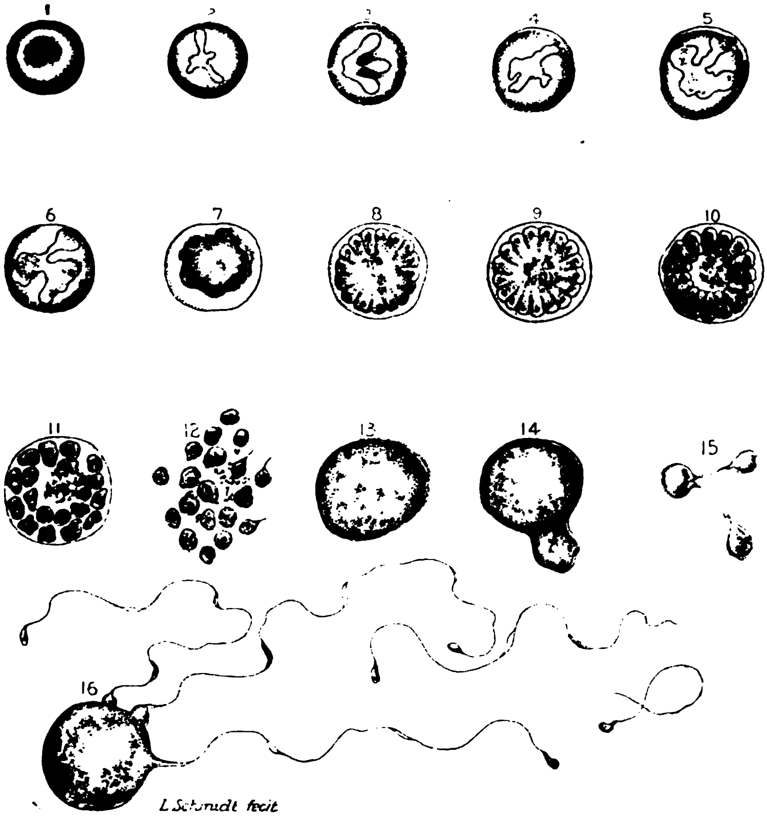
[1] This is an aqueous solution of borax (5 per cent.) and methylene blue (2 per cent.). The blood films are stained with this solution for about thirty seconds; they are then washed in water, dried with filter paper, and afterward by gently warming them over the flame.

PLATE VIII.



The Parasite of Tertian Fever.

1, normal red corpuscle; 2 to 4, non-pigmented stage of the organism, showing ameboid movements; 5 to 7, progressive pigmentation and growth; 8 to 11, process of segmentation; 12, young forms; 13, large extracellular organism; 14, mode of formation of extra-cellular body; 15, small fragmented extra-cellular organism; 16, flagellated body and free flagella. Unstained specimen. (Personal observation.)

2. Pigmented Intracellular Organisms.—These represent a later stage in the development of the parasite, and, like the non-pigmented intracellular bodies, are met with in all types of malarial ever. Their appearance, however, differs considerably in the varius forms. In tertian fever minute granules of a reddish-brown lor appear in the bodies of the organism soon after the paroxysm. hese gradually increase in number, while the invaded corpuscles oportionately become paler and paler, until finally only an indisct, shell-like outline can be discerned. In fresh specimens the nules, which often assume the form of little rods, resembling cteria, exhibit most active molecular movements, attracting attenn at once. The body of the parasite, which during its developnt has increased gradually in size, is probably hyaline, and may ll be seen to undergo ameboid movements. These are not nearly active, however, as in the non-pigmented stage. The movents, moreover, cannot be followed so readily, owing to the presce of the granules. At first sight these appear to be scattered small collections throughout the red corpuscles, and the impression ay be gained that several organisms are present at the same time. pon closer investigation, however, it will be seen that this is only pparently the case, and that the granules are confined to the bulbus extremities of the pseudopodia of a single parasite. Before he end of forty-eight hours the organism has filled out the entire ed corpuscle, which at the same time has attained a larger size han normal. The ameboid movements become less and less marked, nd the pigment granules, which may still be quite active, tend to ollect about the periphery (Plate VIII).

In quartan fever pigmented intracellular bodies likewise appear oon after the paroxysm. The individual granules, however, are omewhat larger, of more irregular size, and darker in color than hose seen in the tertian type (Plate IX, Fig. 2). Instead of exibiting active molecular movements, moreover, they are almost ntirely quiescent, and usually are grouped along the periphery of he organism. While ameboid movements can at first be observed, hese become less and less marked, until finally, at the end of from ixty-four to seventy-two hours, they cease. The organism then resents a round or ovoid form, but does not fill the red corpuscle ntirely. It is curious to note that in this form of ague the red orpuscles do not become decolorized, but rather darker than normally nd at times specimens may be seen which present a distinctly greensh or brassy appearance. When the parasite has become fully leveloped the corpuscle is smaller than normally, and, on staining, t may be seen that the organism still is surrounded by a narrow one of corpuscular protoplasm even when this is not apparent in nstained preparations.

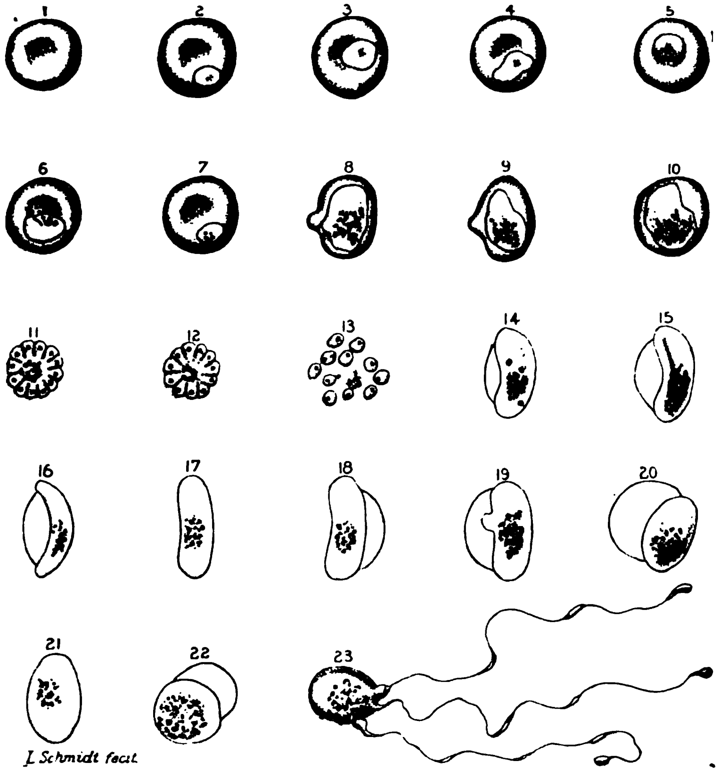
The pigmented intracellular bodies which may be found in estivo-autumnal fever (Plate IX, Fig. 1) can readily be distinguished from those observed in tertian and quartan ague. As in those types, pigment granules also appear after the paroxysm; they are never numerous, however, and often only one or two minute dark granules can be detected near the periphery. The organism, even in the later stages of its development, scarcely ever occupies much more than one-third of the corpuscles. Usually the granules exhibit scarcely any movements. As in the quartan type of ague, decolorization of the red corpuscles does not occur, and here, as there, a greenish, brassy appearance often is observed.

At the beginning and during the paroxysm forms are at times seen in which the few pigment granules that may be present have gathered in the centre of the parasite and formed a solid clump. From the facts that these are observed only during the paroxysm, and that central blocks of pigment are found only during the stage of segmentation (see below) in tertian and quartan ague, Thayer and others conclude that these bodies are presegmenting forms of the parasite. This belief is strengthened by the observation that pigment-bearing leukocytes are then also seen, which in the other types of fever likewise are found only at this time.

3. Segmenting Bodies.—In cases of tertian and quartan fever the process of segmentation may be observed directly under the microscope, if specimens of blood are obtained just prior to or during the chill. In tertian fever organisms will then be seen in which the destruction of the red corpuscles has advanced to a stage in which it is only possible to make out a pale contour of the original host. The parasite itself has gradually assumed a granular appearance, and the pigment granules, which until then have exhibited pronounced molecular movements, now become quiescent, larger and rounder, and show a distinct tendency to collect in the centre of the body. Here they form a roundish mass in which the individual components can scarcely be made out. While this change in the position of the pigment is taking place, beginning segmentation of the surrounding granular protoplasm will be observed. This at first is most marked at the periphery, from which delicate striæ will gradually be seen to extend toward the central mass, dividing up the protoplasm into a number of oval bodies which closely resemble the petals of a flower (Plate VIII). Still later these bodies, which in reality are the sporules (merozoites) of the parasite, will be found scattered in an irregular manner throughout the interior of the organism. The apparent envelope then disappears, and the sporules, which in tertian fever usually number from fifteen to twenty, lie free in the blood. Quite frequently, also, a sudden expulsion of the little bodies is observed and the impression gained as though the envelope had been burst asunder. Upon closer inspection, even at the petal stage, it will be seen that almost every sporule presents a tiny dot in its interior,

PLATE IX.

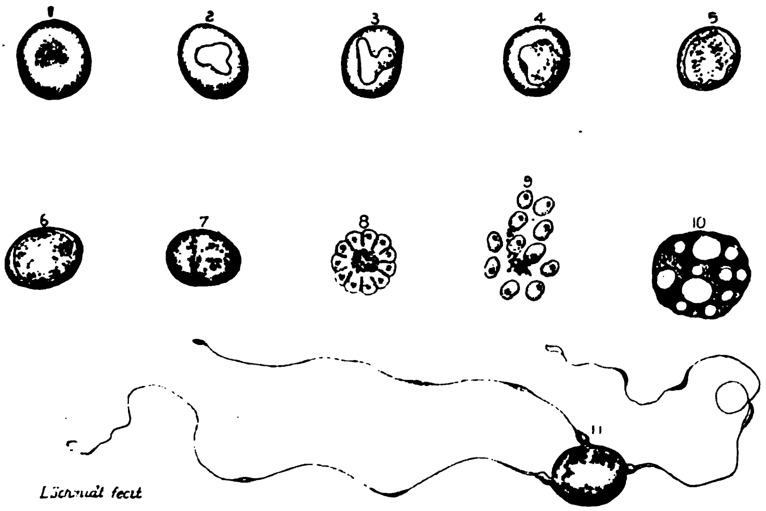
FIG. 1.



The Parasite of Estivo-autumnal Fever.

1, normal red corpuscle; 2 to 10, gradual growth of the organism; 11 and 12, segmenting bodies 13, young forms; 14 to 22, crescents, ovoids and spherical bodies, with and without bib; 23, flagellated body. Unstained specimen. (Personal observation.)

FIG. 2.



The Parasite of Quartan Fever.

1, normal red corpuscle; 2 to 6, gradual growth of the organism; 7, pigmented extracellular body; 8, segmenting body; 9, young forms; 10, vacuolated extracellular body; 11, flagellated form Unstained specimen. (Personal observation.)

which may at first sight be mistaken for a pigment granule, but which in all probability is a nucleus. After the expulsion of the sporules these are frequently seen to move about in an active manner, but sooner or later they come to rest.

While the progress of segmentation is very frequently observed to proceed in the manner described, this is not invariably the case. It may thus happen that segmentation occurs before the pigment granules have had time to gather at the centre, or that the parasitic protoplasm breaks up into sporules directly without the intervention of the petal stage. In every case, however, the formation of sporules is associated directly with the occurrence of a paroxysm and represents the asexual type of reproduction of the parasite (schizogony).

The sporules, unless destroyed by leukocytes, in turn invade new corpuscles, cause their destruction, and become segmented, thus giving rise to a new generation. As the process of segmentation coincides in time with the occurrence of the chill, it is apparent that the interval elapsing between two consecutive chills—*i. e.*, the type of the ague—depends upon the rapidity with which the organisms arrive at maturity.

In quartan ague segmentation differs somewhat from that observed in the tertian form. It will here be observed that the pigment granules, which have gathered along the periphery of the organism, as the parasite approaches maturity become arranged in a stellate manner, and apparently reach the centre through definite protoplasmic channels. Here they form a dense clump, and while the protoplasm assumes a finely granular appearance, segmentation proper begins and proceeds as in the tertian form. The number of segments, however, is smaller, varying between six and twelve. The entire segmenting body, moreover, is smaller than in the tertian form, and the segments are arranged in a more symmetrical manner. Here, indeed, the most perfect rosettes are observed (Plate IX, Fig. 2).

In estivo-autumnal fever segmenting bodies are only exceptionally seen in the peripheral blood, and it appears that the process of reproduction occurs principally in the spleen. The segments, as a rule, number from ten to twenty. The segmenting body itself, however, is much smaller than in either the tertian or quartan form, and it is not possible to distinguish any remains of the original host.

4. Crescents, Ovoids, and Spheroids (Plate IX, Fig. 1).—These are observed only in cases of estivo-autumnal fever when this has persisted for at least a week. At first sight they apparently bear no relation to the other forms which have been described, but it is known that they are derived directly from the pigmented intracellular forms. Specimens may thus be met with in which crescentic bodies are found in the interior of red corpuscles that have lost but little of their original color. Such observations, however, are not common. The typical crescents which are usually seen are

highly refractive bodies, somewhat larger than a red corpuscle, measuring from 7 μ to 9 μ in length by 2 μ in breadth. Their extremities are usually rounded off and joined by a delicate, curved line bridging over their concave border. This is supposed to represent the remains of the original host. At other times this hood-like appendage is found along the convex border. The little pigment granules and rods, which are always found in the interior of the crescents, are generally collected about the centre of the body, but they are occasionally also seen in one of the horns. While usually quiescent, a migration of some of the granules toward one extremity and back to the central mass may be observed. The ovoid and spherical bodies, which are usually smaller than the crescents, exhibit the same general features and often are provided likewise with a little hood. It is now known that the spherical bodies develop from the ovoids, and these again from the crescents.

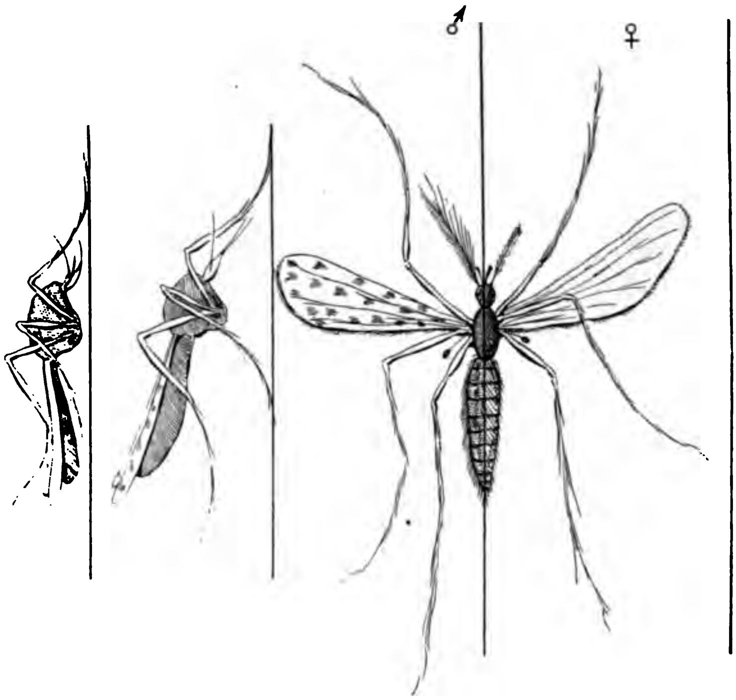
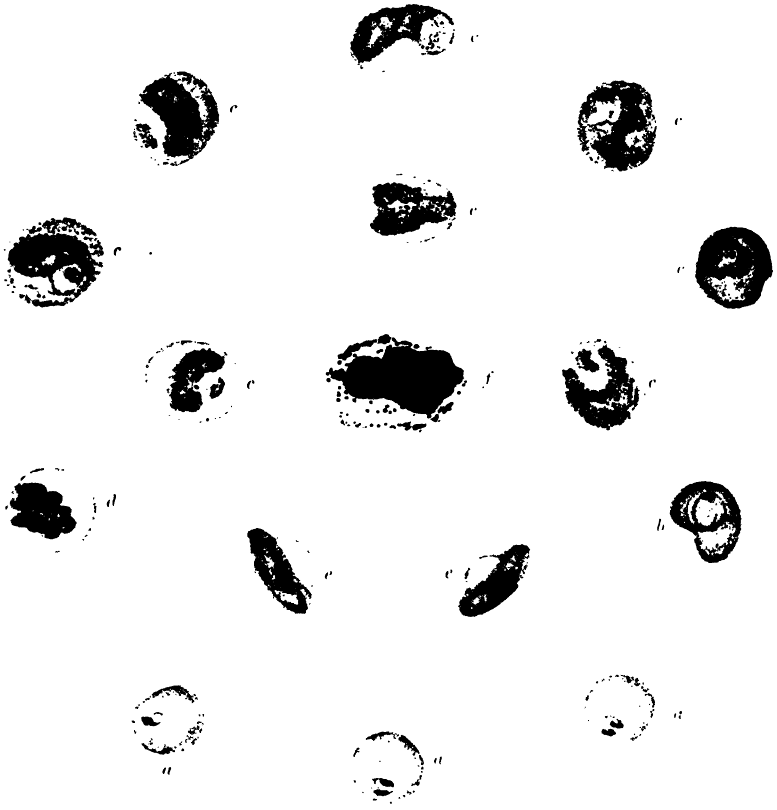


FIG. 45.--(From Doflein.)

5. Extracellular Pigmented Bodies or Gametes.—In tertian and quartan ague some of the pigmented intracellular bodies, instead of undergoing segmentation when they have arrived at maturity, leave their hosts and appear as such in the blood. Some of them

PLATE X.



Malarial Organisms.

a, young estivo-autumnal ring bodies; *b*, young tertian form; *c*, tertian parasites in various stages of development; *d*, segmenting organism; *e*, estivo-autumnal crescents; *f*, large mononuclear leukocyte carrying pigment from ingested malarial organisms. Stained with Wright's stain.

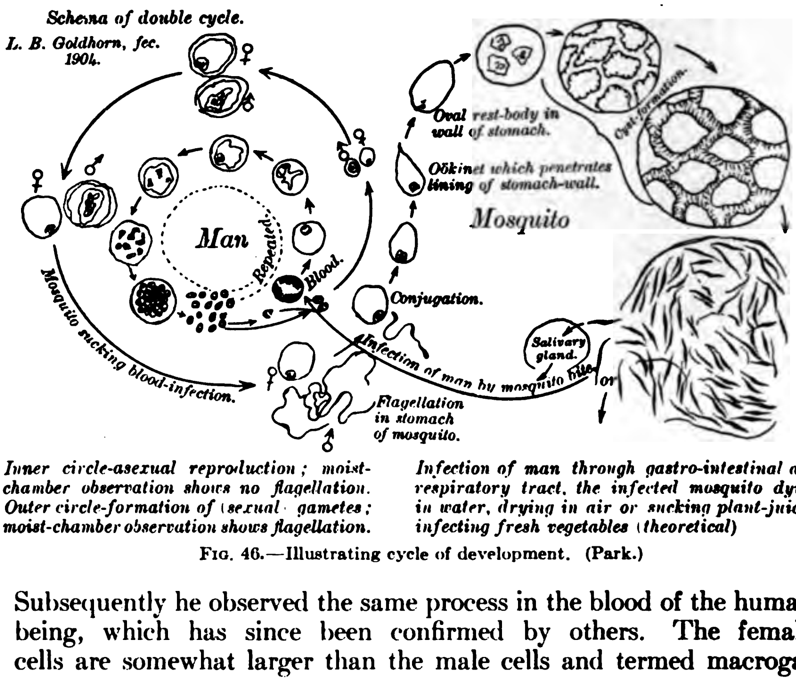
t the same time increase considerably in size, and in the tertian
rm may become as large as a polynuclear leukocyte (Plate VIII).
he pigment granules, moreover, exhibit an activity in their move-
ents which is most astonishing and never observed under other
onditions. The outline of the parasite is then usually irregular
nd quite indistinct. Upon careful observation it will be seen
hat in some of these bodies the movements of the granules after a
hile become less and less marked, and finally cease, while the body
f the parasite itself becomes still more irregular in outline. This
ppearance is undoubtedly referable to the death of the organism.
n others a gradual fragmentation is observed, small particles of the
igmented mother-substance being cut off from the parent form.
t is thus quite common to see the original parasite break up into
ur or five smaller bodies, in which the movements of the pigment
ranules persist for some time. Sooner or later, however, even these
ease, the outlines of the bodies become more and more indis-
nct, and death occurs. In still others the formation of vacu-
les may be observed, the pigment granules at the same time
ecoming quiescent. This process is likewise regarded as one of
egeneration. Most interesting, however, is the fact that *flagel-
tion* may occur in some of these extracellular forms. This may
ometimes be hastened in the wet specimen by gently breathing
pon the slide so as to form a thin film of moisture It will then
e observed that the pigment granules which exhibit a most sur-
rising activity tend to collect near the centre of the organism, while
t the same time curious undulating movements may be made out
long its contours. Suddenly one or more (one to six) slender fila-
ents will be seen to protrude from as many points on the periphery,
resenting minute enlargements here and there in their course (Plate
X) (polymites). The length of these filaments, or flagella, as
hey are termed, varies considerably. As a rule, it does not exceed
he diameter of from five to eight red corpuscles, but longer speci-
ens are at times observed. With these flagella the organism makes
ost active whipping movements, scattering the red corpuscles to
e right and left. Attention is, indeed, usually first drawn to the
resence of these bodies by the disturbance which they cause in the
eld of vision. Occasionally one of the flagella may be seen to become
etached from the body of the parasite and to move rapidly about
mong the corpuscles in a snake-like manner. In microscopic speci-
ens they gradually come to a rest and often curl into a spiral.

Beyond the fact that the flagellate organisms in tertian fever are
rger than in the quartan form, no special points of difference exist
Plate IX, Fig. 2).

In estivo-autumnal fever similar changes may be observed. The
agellate forms are here direct derivatives of the crescents, which have
anged to ovoids and these to spheroids. The flaggellates, as in

quartan fever, are smaller than those observed in the tertian form (Plate IX, Fig. 1).

The significance of the flagellate organisms is now well understood. They represent the male element in the sexual reproduction of the malarial parasite (microgametocytes) and the beginning of a cycle of development, which takes place outside of the human body, in the bodies of mosquitoes of the species Anopheles. The beginning of this cycle was first observed by MacCallum in the blood of infected crows. He here discovered that when one of the flagella (microgametes) broke loose it almost always sought out another full-grown form of the parasite which had not undergone segmentation, and penetrated this, just as the spermatozoon penetrates the ovum.


Schema of double cycle.
L. B. Goldhorn, fec. 1904.
♀
♂
♀
♂
Man
Repeated.
Blood.
Mosquito sucking blood-infection.
♀
Flagellation in stomach of mosquito.
♂
Conjugation.
Oökinet which penetrates lining of stomach-wall.
Oval rest-body in wall of stomach.
Cyst-formation.
Mosquito
Salivary gland.
Infection of man by mosquito bite.
or

Inner circle-asexual reproduction; moist-chamber observation shows no flagellation. Outer circle-formation of (sexual) gametes; moist-chamber observation shows flagellation.

Infection of man through gastro-intestinal and respiratory tract, the infected mosquito dying in water, drying in air or sucking plant-juices, infecting fresh vegetables (theoretical)

FIG. 46.—Illustrating cycle of development. (Park.)

Subsequently he observed the same process in the blood of the human being, which has since been confirmed by others. The female cells are somewhat larger than the male cells and termed macrogametes. The further development (sporulation) of the fertilized forms (oökinetes), however, does not take place in the human blood, but in the mosquitoes. The fertilized organism penetrates the stomach wall of the insect and here gives rise to the formation of little cysts (oöcysts) in which after about seven days numerous irregular, rounded, ray-like striæ appear. After a time the capsules of the cysts burst and the delicate, thread-like bodies (the sporozoites) are set free in the body cavity of the mosquito, and shortly after appear in the salivary glands. These bodies represent the young parasites, which result from the sexual reproduction of the adult organism. If at this stage of their development the infected mosquito is allowed to bite a human being malarial infection results, with the appearance in the blood of the hyaline forms already described.

From the above description it will be seen that three forms of the malarial parasite may be found in the blood, viz., the parasite of tertian, quartan, and estivo-autumnal fever, and it has been shown that these forms may readily be distinguished from each other. In tertian and quartan fever several groups of the same organism may be present at one time, and as the process of segmentation coincides with the occurrence of a paroxysm it will readily be seen that the number of paroxysms within a given time depends upon the number of groups which may be present in the blood. If a double infection with the tertian parasite has occurred, one group of organisms may thus have just reached the segmenting stage, while the second group has attained only a twenty-four hours' growth, the result being that maturity is reached by the two groups on successive days. Quotidian fever is then the result. Should still other groups be present, the clinical picture will accordingly become more complicated. In quartan ague, similarly, double quartan fever will occur if two groups are present, and triple quartan fever if three groups are present at one time. Mixed infections, further, are also possible.

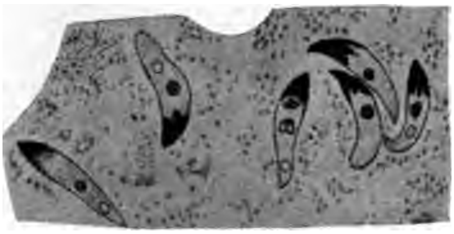


FIG. 47.—Oökinetes of pernicious parasites in the stomach of Anopheles maculipennis thirty-two hours after having been sucked in. (Grassi.)

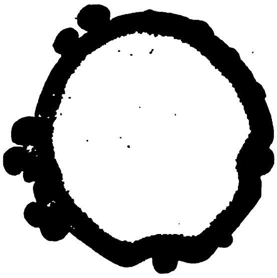


FIG. 48.—Transverse section of the stomach of an anopheles, with cysts of pernicious parasites. (Grassi.)

PIGMENTED LEUKOCYTES.—In conclusion, it may not be out of place to refer to the presence of pigment-bearing leukocytes in the blood of malarial patients. (See Plate X.) These are quite constantly met with during the paroxysm, and it is indeed often possible to

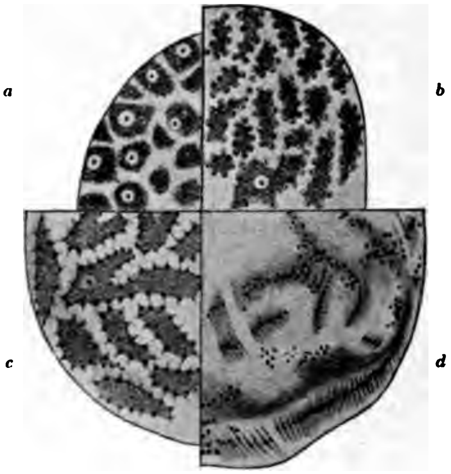


FIG. 49.—Four stages of sporulation of malarial parasites from Anopheles maculipennis, strongly magnified: *a–c*, the pernicious parasite; *a*, four to four and a half days after ingestion; *b* and *c*, five to six days after ingestion; *d*, tertian parasite, eight days after ingestion. (Grassi.)

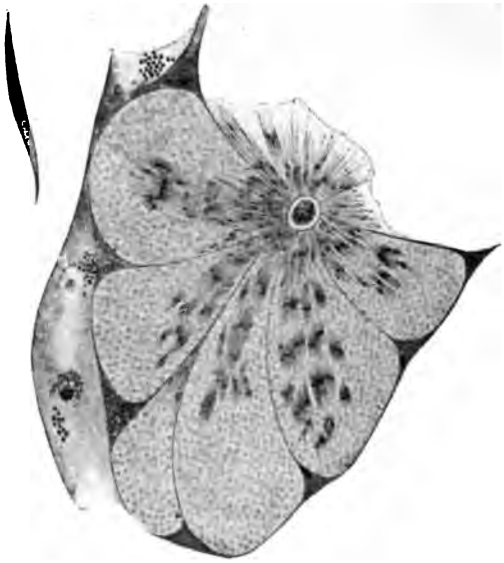


FIG. 50.—Section through a tubule of the salivary gland of an anopheles, with sporozoites of the pernicious parasites; above an isolated sporozoite with higher magnification. (Grassi.)

observe the process of *phagocytosis* directly under the microscope. The forms which are taken up are the small, fragmented, extracellular forms, the flagellate bodies, segmenting bodies, and free pigment clumps. In every case where pigment-bearing leukocytes are observed, malarial fever should be suspected and a careful examination made, as a melanemia only occurs in this disease, in relapsing fever, and in connection with the rare melanotic tumors, in which not only leukocytes containing melanin may occur in large numbers, but also masses of pigment floating free in the blood.

LITERATURE.—A. Laveran. Nature parasitaire des accidents de l'impaludisme, Description d'un nouveau parasite, Paris, 1881. P. Manson, Tropical Diseases, Cassell & Co., London, 1900, p. 1. For a full account of the literature, see the monograph by W. S. Thayer and J. Hewetson, "The Malarial Fevers of Baltimore," Johns Hopkins Hosp. Rep., vol. v. On recent advances in our knowledge concerning the etiology of malarial fever, see W. S. Thayer, Phila. Med. Jour., 1900, p. 1046, where a full account of the literature is given. T. B. Futcher, "A Critical Summary of Recent Literature concerning the Mosquito as an Agent in the Transmission of Malaria," Amer. Jour. Med. Sci., 1899, p. 318. W. S. MacCallum, "On the Hematozoon Infection of Birds," Jour. Exper. Med., vol. iii, p. 117. E. L. Opie, "On the Hematozoon of Birds," ibid., p. 79. F. Grohe, "Zur Gesch' d. Melanaemie" Virchow's Archiv, 1861, vol. xx, 306.

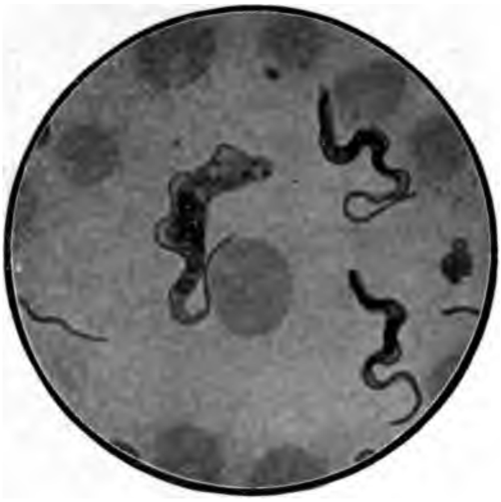


FIG. 51.—Trypanosoma gambiense (sleeping sickness) in blood of a rat. Two types are shown; the broad, pale form (female?) is dividing. Magnification 1500 times. MacNeal's stain. (From Novy.)

Trypanosomiasis.—The first authentic report concerning the occurrence of trypanosomiasis in man was made by Dutton in 1902, while in animals their occasional presence had long been recognized (frogs, rats, dogs, groundhogs, etc.). In tropical regions certain species are pathogenic for certain domestic animals. The tse-tse fly disease or Nagana of Africa, the Surra disease of Asia, and the mal de caderas of South America are all referable to infection with trypanosomes (observed in the horse, the African buffalo, the ox, the donkey, mule, antelope, camels, and elephants). Especially interesting is the observation of Castellani and Bruce of the association of trypanosomiasis with a certain symptom-complex, of which the so-called sleeping sickness is one of the possible manifestations. Bruce could demonstrate the organism in the blood of 12 out of 13 cases, and in the cerebrospinal fluid in all of 38 cases. The findings of these earlier observers have since been abundantly confirmed, and it is now generally conceded that the disease in question is referable to infection with trypanosomes.

The *Trypanosoma gambiense* (Dutton) is from 8 to 25 μ long, and from 2 to 2.8 μ broad. It is provided with an undulating membrane and a flagellum, which starts from a centrosome or micronucleus lying in the posterior end of the animal, and projects somewhat beyond the anterior end. (See Figs. 51 and 52.) There is an oval nucleus which is centrally located and is made up of chromatin granules.

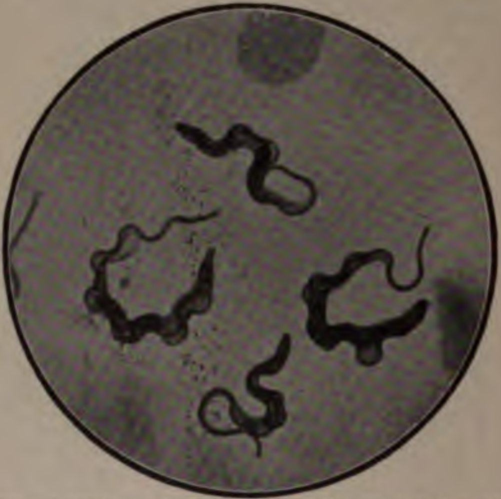


FIG. 52.—Trypanosoma gambiense from same preparation as preceding, showing the usual form; some cells in process of division. Magnification 1500 times. (From Novy.)

In the wet preparation the organism exhibits slow spiral movements. It is found free in the blood plasma, but may also be seen in the interior of leukocytes, which latter manifestly destroy the organisms exactly as the malarial parasites. In dry specimens the trypanosomes can be readily stained with any basic dyes; with the Romanowsky stain or one of its modifications it is stained like the malarial organism. Levaditi[1] recommends the following method as especially valuable: Fixation in absolute alcohol and ether for five minutes; primary staining for two minutes with a saturated solution of Bismarck brown, followed by washing and counterstaining with

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[1] Soc. de biol., November 23, 1903.

Unna's polychrome blue (diluted one-half with water) for two minutes. The specimens are rinsed in water, dried over a flame, and examined as usual.

The number of organisms in a blood preparation is rarely large; as a rule, not more than 3 to 8 are found to a cover-slip. During apyrexia they are not seen.

Infection in man probably occurs through a biting fly—the Glossina palpalis, which supposedly transmits the disease in a purely mechanical way.

Novy and McNeal succeeded in cultivating the trypanosoma of Bruce in the water of condensation from a medium of agar mixed with defibrinated rabbit's blood (1 to 1) at 25° C., and the rat trypanosome (Trypanosoma lewisi) in a similar medium containing 1 part of blood for 2, 5, or even 10 parts of agar.

LITERATURE.—Dutton, Thompson-Yates Laboratory Rep., 1902, vol. iv, part ii, p. 455; and Brit. Med. Jour., 1903, vol. i, p. 304. Castellani and Bruce, ibid., pp. 1218 and 1431; Jour. Trop. Med., 1903, p. 167. Novy and McNeal, Journ. Amer. Med. Assoc., November 21, 1903. Novy, ibid., Jan. 5, 1907.

Relapsing Fever.—Relapsing fever is characterized by the presence in the blood, and here only, of spirochetes which bear the name of their discoverer, Obermeier. In order to search for the organisms no special precautions are necessary. After having cleansed the finger a drop of blood is mounted on a very thin cover-glass. This is inverted directly upon a slide, when the specimen is ready for examination; an oil-immersion lens is not required. Attention is drawn to the presence of the organisms by disturbances which are noticeable among the red corpuscles, and upon careful examination it will be seen that these are caused by the wriggling movements of the spirochetes. The Spirochætæ Obermeieri are long, slender filaments, measuring from 36 μ to 40 μ in length by 0.3 μ to 0.5 μ in breadth, and present from eight to twelve incurvations of equal size with tapering extremities. These two last characteristics serve to distinguish this species from that described by Ehrenberg, in which the radius of the incurvations is not the same in all, and in which the extremities do not taper (Fig. 53).

The number of spirilla which may be found in a drop of blood varies, being greater during the access of the fever, when twenty, or even more, may be observed in the field of the microscope. They occur singly or in bunches of from four to twenty. In the quiescent stage they are arranged sometimes in the form of rings or of the figure 8. After the crisis they seem to disappear entirely, and their presence during an afebrile period may therefore be regarded as indicating a pseudocrisis. During the afebrile periods small, bright, round bodies have been described in the blood, which according to some are spores, but according to others represent merely debris of the spirilla.

Culture experiments have not been very satisfactory, although Koch observed an increase in their number at a temperature of from 10° to 11° C.

Koch has shown that in African relapsing fever, which is likewise due to a spirocheta, infection occurs through the bite of a certain tick, Ornithodorus moubata, which acts as intermediary host in the development of the organism.

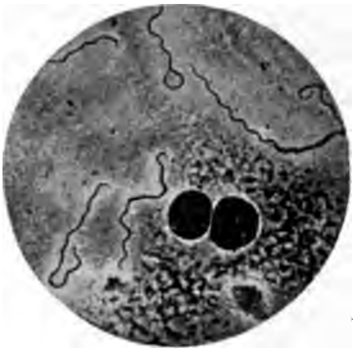

FIG. 53. Spirochætæ Obermeieri; blood smear. × 1000 diam. (From Itzerott and Niemann.)

The tick fever of the Congo Free State is apparently identical with the African recurrens described by Koch. Infection likewise occurs through the bite of infected ticks, Ornithodorus moubata.

Hödlmoser has shown that the blood of recurrens is spirilla agglutinating. But as the culture of the organisms is practically not possible, the blood of a second case must be available for the test.

LITERATURE. Heidenreich. Untersuch. über d. Parasit. d. Rückfallstyphus, Berlin, 1877. Moczutkowsky, Deutsch. Arch. f. klin. Med., vol. xxiv, p. 80, and vol. xxx, p. 165. Blisener, Inaug. Diss., Berlin, 1873. Engel, Berlin. klin. Woch., 1873, p. 409. J. E. Dutton and J. L. Todd, Brit. Med. Jour., November 11, 1905. 76 Versammlung d. Nat. u. Azt., Breslau, 1904.

Typhus Fever.—According to Gottschalk[1] a protozoon, closely related to Pyroplasma bigonicum, which he terms Apiosoma, can be demonstrated in the blood of typhus fever. He claims to have found sporulation cysts and flagellated forms. Infection according to Gottschalk may occur through bedbugs.

Tropical Splenomegaly (Kala-azar).—Through the researches of Donovan, Leishman, and Ross especially it has been established that in tropical splenomegaly (cachexial fever, Kala-azar) parasites may be demonstrated in the blood which are probably etiologically connected with the pathological condition. The organism in question has been termed the Leishmania Donovani (Leishman-Donovan body, Cunningham-Leishman-Donovan body). It represents a stage

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[1] Deutsch. med Woch., 1903, No. 19.

PLATE XI.

Leishmania-Donovani.

[illegible] of leukocytes undergoing dissolution. (Stained with Leishman's stain.

in the development of a trypanosome, as was suggested by Rogers and as has since been shown by cultural experiments by Leishman and Statham.

In the *peripheral* blood the organisms are rarely found and only when the temperature is high. Splenic puncture gives the best results. Donovan suggests that it is well to keep the patient flat on the back for twenty-four hours after the operation and to give a dose of calcium chloride immediately after and twice again at intervals of three hours (to prevent hemorrhage). The parasites are principally met with in large mononuclear cells. The typical forms are oval or circular with a well-marked contour (Plate XI). There is a deeply staining nucleus lying against the capsule and a deeply staining, rod-like centrosome. They may occur singly or in pairs or in zoöglœa masses. They are readily stained with any one of the methylene-azure mixtures (Hastings, Giemsa, Leishman, etc.).

LITERATURE.—R. Ross, Brit. Med. Jour., July 9, 1904. L. Rogers, Lancet, July 23, 1904. Leishman and others, Discussion, Brit. Med., Jour., September 17, 1904. Leishman and Statham, Jour. Royal Army Med. Corps, March, 1905.

Syphilis.—The Spirocheta pallida (Treponema pallidum) has been demonstrated in the blood during life. Under ordinary circumstances, however, its search is here not likely to be attended by success. For diagnostic purposes it should be looked for in scrapings from chancres, papules, condylomas, in the aspirated juice of enlarged lymph glands, etc. (For a description of the organism see Examination of Syphilitic Material.)

Spotted Fever.—In the so-called spotted fever, which occurs in Montana, Nevada, Oregon, etc., an intracorpuscular ameboid, nonpigmented organism has been described by Wilson and Chowning, as also by Anderson, which they regard as the cause of the disease. They term this the Pyroplasma hominis. Infection supposedly takes place through ticks belonging to the species Dermacentor reticulatus.

I have studied the blood in several cases which were placed at my disposal by Drs. McCalla, Maxey, and Pease, but was unable to find such structures. Craig and Stiles express themselves in a similar manner.

LITERATURE.—Wilson and Chowning, Jour. Amer. Med., Assoc., 1902, vol xxxix, p. 131. J. F. Anderson, Amer. Med., 1903, vol. vi, p. 506. Craig, Amer. Med., December 10, 1904.

Filariasis.—According to Manson, the embryos of at least four, and possibly five and even more distinct species of nematodes may be found in the blood of man. These various blood worms Manson designates as the Filaria nocturna, Filaria diurna, Filaria perstans, Filaria demarquaii, Filaria ozzardi (a doubtful species), and a sixth, which may or may not be connected with one of the two last, the

Filaria magelhæsi. Two of these at least are of pathological import, viz., the Filaria nocturna and the Filaria perstans.

Filaria Nocturna (Manson): *syn.*, Filaria sanguinis hominis (Lewis). This filaria is the embryo form of the Filaria Bancrofti (Cobbold), which inhabits the lymphatics and is unquestionably the cause of endemic chyluria, of various forms of lymphatic varix, of tropical elephantiasis arabum, and possibly also of other obscure tropical diseases. The organism in question is widely distributed. It is indigenous in almost all tropical and subtropical countries as far north as Spain in Europe and Charleston in the United States, and as far south as Brisbane in Australia. It is very common in Cochin and in some of the South Sea Islands, where one-third and one-half of the population, respectively, appear to be infected.

In the following description of both parent and embryo form I quote largely from Manson's account of the parasite in his admirable manual of tropical diseases.

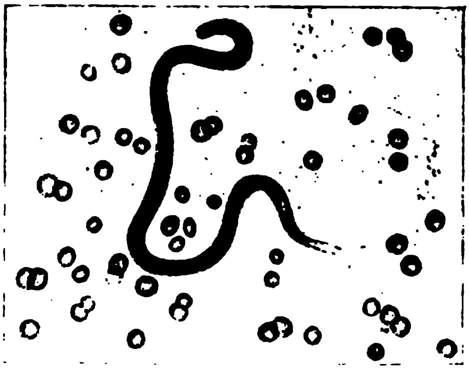

Fig. 54.—Filaria sanguinis.

The parent filarias are hair-like, transparent worms measuring from 7.5 to 10 cm. in length. The sexes live together, often inextricably coiled about each other. Sometimes they are enclosed, coiled several in a bunch, and tightly packed in little cyst-like dilatations of the distal lymphatics; sometimes they lie more loosely in lymphatic varices; sometimes they inhabit the large lymphatic trunks between the glands, the glands themselves, and probably not infrequently the thoracic duct. The female is the larger; there are two uterine tubes which occupy the greater part of the body, and which are filled with ova in various stages of development. The vagina opens near the mouth; the anus just in advance of the tip of the tail. The cuticle is smooth and without markings. In both sexes the mouth end tapers slightly; it is clubbed and simple. The male is characterized by its marked disposition to curve. The cloaca gives exit to two slender, unequal spicules.

In the wet preparations the *Filaria nocturna* appears as a transparent, colorless little worm, which wriggles about most actively, constantly agitating and displacing the corpuscles in its vicinity. It will be noticed, however, that the animal does not propel itself through the drop of blood, but remains stationary. At first the movements are so active that it is impossible to make out any anatomical details; after a number of hours, however, the movements become more sluggish, and it is then possible to study the worm with more ease. It measures about 0.31 mm. in length by 0.007 to 0.008 mm. in width. With the higher power it will be seen that the entire worm is enclosed in a delicate envelope, in which it moves backward and forward, the sheath being much larger than the worm (Fig. 54). It is owing to the presence of this sheath that active locomotion on the part of the worm is not possible. About the posterior part of the middle third of the parasite there is an irregular aggregation of granular matter, which represents a viscus of some sort. With a high power one can further make out a delicate transverse striation in the musculocutaneous layer throughout the entire length of the animal. In stained specimens two V-shaped light-spots can be made out: one at a point about one-fifth of the entire length of the organism, backward from the head end; the other, very much smaller, a short distance from the tail. The first Manson designates the "V" spot, the second the tail spot. In stained specimens these two spots are readily made out, as they do not take the color. When the movements of the animal have almost ceased, one can see on careful focussing that the head is constantly being covered and uncovered by a six-lipped or hooked and very delicate prepuce; and, moreover, one can sometimes see a short fang of extreme tenuity suddenly shot out from the uncovered extreme cephalic end, and as suddenly retracted.

TECHNIQUE.—The examination should be made late in the evening, after the patient has rested for a number of hours. Drops of blood are then mounted, wet, on slides and ringed with vaselin to prevent the specimen from drying. In such preparations the filarias keep alive for a week or longer. They should be searched for with a low power—an inch objective is very convenient for the purpose. Attention is directed to their presence by the commotion which they cause among the neighboring blood corpuscles.

To prepare permanent mounts blood smears are best made on slides, which are then stained with eosinate of methylene blue in the usual manner. Working with the blood of infected animals, I have thus obtained very good results. The V and tail spots are very well brought out. To show anatomical details, however, staining with eosin and hematoxylin, after fixing the smears with alcohol, gives the best results; in this manner the sheath is very well shown, as also the structure of the musculocutaneous layer.

The number of worms which may be found in a specimen is very variable. During the daytime they are rarely seen, and, if at all, only one or two specimens at most are found. As evening approaches, however, commencing about 5 or 6 o'clock, the filarias enter the peripheral circulation in increasing numbers. At midnight the maximum number is about reached, with from 300 to 600 to the drop of blood. Later they gradually decrease, and by 8 or 9 A.M. they have again disappeared. This periodicity, however, may be reversed if the patient is made to sleep during the daytime and remains awake at nights. During their absence from the peripheral circulation they may be found in the larger arteries and in the lungs.

In non-active cases the number of filarias even at night is quite small. In one instance of this kind I found only the sheath of a single worm while examining perhaps fifty specimens.

Infection occurs through the females of mosquitoes belonging to both the culex and anopheles family which have fed on the blood of filaria-infected individuals. The history of the parasite while in the body of the mosquito is in brief the following: After their arrival in the stomach the young worms shed the sheath and invade the thoracic muscles, where they increase in size (to 1.5 mm.), develop a mouth, an alimentary canal, and a trilobed tail. They then find their way into the abdomen, where, in suitably prepared sections, they may occasionally be seen in the tissues about the stomach, and even among the eggs in the posterior part of the abdomen. The majority now find their way to the base of the proboscis and under appropriate conditions out through the proboscis by a channel which they make for themselves. After introduction into the human body the organism finds its way into the lymphatics, where it attains sexual maturity; fecundation takes place and the new generation of filarias enter the blood current by way of the thoracic duct and the left subclavian vein. The development of the embryo form in the mosquito occupies from sixteen to twenty days.

Whether or not infection can occur in any other way is not known. We could conceive that some of the worms are eliminated with the eggs of the mosquitoes, and that infection could then take place through contaminated drinking water.

Filaria Perstans.—This species is of interest, as it was thought to be concerned in the causation of the so-called sleeping sickness of west tropical Africa. It has likewise been found in the Buck Indians of British Guiana, among whom the same sickness also occurs.[1] The organism observes no periodicity, but is present in the blood both during the daytime and at night.

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[1] More recent observations tend to throw doubt on this relationship and rather suggest a connection between a species of trypanosoma and sleeping sickness. (See p. 187.)

The embryo worm is smaller than the Filaria nocturna; it measures about 0.2 mm. in length by 0.004 mm. in breadth. It has no sheath, and its caudal end is truncated and abruptly rounded. There is no hooked cephalic prepuce. Its motion is progressive.

The adult form measures 70 to 80 mm. in length. The tail in both sexes is incurvated and the chitinous covering at the extreme tip split, as it were, into two minute triangular appendages. They have been found in the connective tissue, at the root of the mesentery, behind the abdominal aorta, and beneath the pericardium.

LITERATURE.—Mosler u. Peiper, Spezielle Pathol. u. Therap., 1894, vol. vi, p. 219. P. Manson, Allbutt's System of Medicine, vol. ii. I. Guitéras, Med. News, April, 1886. F. P. Henry, ibid., 1896. E. Opie, Amer. Jour. Med. Sci., 1901, vol. cxxii, p. 251. P. Manson, Tropical Diseases, Cassell & Co., London, 1900.

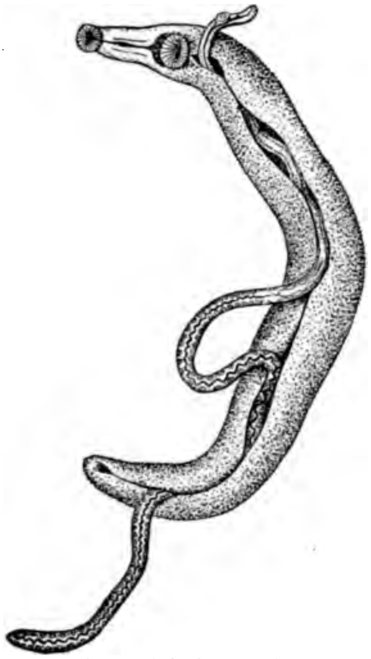

FIG. 55.—Male and female specimens of the human blood fluke (*Bilharzia hæmatobia*). × 12. (After Looss.)

Distomiasis (Bilharziasis).—Bilharzia hæmatobia (Cobbold): *syn.*, Gynæcophorus (Diesing); Distomum hæmatobium (Bilharz); Schistosoma hæmatobium (Weinland); Distoma capense (Harley); Thecosoma (Maguin-Tandon).

The Bilharzia hæmatobia belongs to the class of trematode platodes. According to Bilharz, the greater portion of the Fellah and Coptic population of Egypt is infected. It is abundant in South Africa, and has also been observed in Mesopotamia, and apparently in Arabia. In the United States a few isolated cases have been seen which were undoubtedly imported. From Europe no endemic cases have been reported. The parasite may give rise to diarrhea, hematuria, and ulceration of the mucous surfaces.

The male is smaller but thicker than the female, measuring from 12 to 15 mm. in length by 1 mm. in breadth. On its abdominal surface a deep groove is found with overlapping edges, which serves for the reception of the female (Fig. 55). It has an oval and a ventral sucker placed close together.

The adult parasites are found in the blood of the portal vein, in its mesenteric and splenic branches, and in the vesical, uterine, and hemorrhoidal veins; they have also been found in the vena cava and may possibly occur elsewhere in the circulation. The eggs are more often seen. They are oval bodies, measuring 0.16 mm. in length by 0.05 mm. in breadth, and are provided with a distinct, spike-like projection which issues from one extremity or the side (Fig. 56). Infection usually takes place through unfiltered drinking water, but may also occur through the skin. Through the portal system the parasite then invades the urogenital system, the anus, and rectum, and may also proliferate abundantly in the intestine, the liver, kidneys, etc. The diagnosis is usually made by examination of the urine, in which the ova will be found.

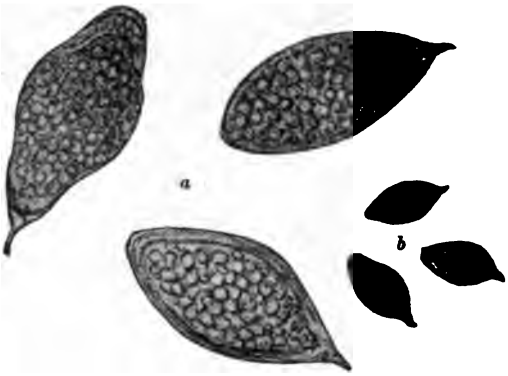

FIG. 56.—Bilharzia eggs from the urine: Group *a* was drawn to scale with B. & L. $\frac{1}{6}$ obj., and 1 in. ocular; group *b* represents their appearance with B. & L. $\frac{2}{3}$ obj.

Another variety of blood fluke has been described by J. Catto,[1] *Schistosoma Cattoi*; it was found in a Chinese who had died of cholera.

LITERATURE.—Bilharz, Wien. med. Woch., 1856, vol. vi, p. 49. Meissner, Schmidt's Jahrbuch., 1882, vol. xx, p. 193. Rütimeyer, Verhandl. d. Cong. f. inn. Med., 1822, vol. xi, p. 144.

Anguilluliasis.—In 1895 Teissier reported a case of intermittent fever in which numerous embryos of anguillula were found in the blood. They disappeared after expulsion of the parasites from the intestinal tract, and at the same time the fever ceased. It is a question, however, whether Teissier's parasite was identical with the common form described by Bavay, Normand, Grassi, and others. Unlike the embryos developing from the eggs of both parasitic and free-living generations, Teissier's form did not present the characteristic double œsophageal enlargement, and he reports, moreover, that in the case of the adult male only one, instead of two, spicules was noted. This view is strengthened by the observation that after inoculation into frogs the worms developed in the intestinal canal and the lungs into giant forms, which may have been *Ascaris nigrovenosa* (*syn.*, Rhabdonema nigrovenosum).

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[1] Brit. Med. Jour., January 7, 1905.

LITERATURE.—Teissier, Compt.-rend. de l'Acad. des sci., 1895, vol. cxxi, p. 171. Arch. de méd. expér. et d'anat. path., 1895, vol. vii, p. 675; ibid., 1896, vol. viii, p. 586.

CHAPTER II.

THE SECRETIONS OF THE MOUTH.

SALIVA.

NORMAL saliva is a mixture of the secretions derived from the submaxillary, sublingual, parotid, and mucous glands of the mouth. It is a colorless, inodorous, tasteless, somewhat stringy and frothy liquid, and serves the purpose of aiding in the acts of mastication, deglutition, and digestion. The quantity secreted in twenty-four hours amounts to about 1500 grams.

General Characteristics.

Normal saliva has a specific gravity of 1.002 to 1.009, corresponding to 4 to 10 grams of solids. The reaction is alkaline, the degree of alkalinity corresponding to from 0.006 to 0.048 per cent. of sodium hydrate. Normally an acid saliva is observed only in newly born infants and in sucklings.

The reaction of the tongue and the mucous membrane lining the mouth is quite commonly acid early in the morning owing to the production of lactic acid by some of the bacteria which are constantly present in the mouth. This acid corrodes the enamel of the teeth, and may ultimately produce dental caries.

Chemistry of the Saliva.

In order to give an idea of the general composition of the saliva the following analyses are appended; the figures correspond to 1000 parts by weight:

Water	995.20	994.20	988.10
Ptyalin[1]	1.34	1.30	1.30
Mucin Epithelium	1.62	2.20	2.60
Fatty matter	...	..	0.50
Sulphocyanides	0.06	0.04	0.09
Alkaline chlorides	0.84		
Disodium phosphate	0.94	2.20	3.40
Magnesium and calcium salts	0.04		
Alkaline carbonates	traces.		
Nitrites	traces.		

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[1] These figures are too high, as they refer to the total precipitate obtained with alcohol.

In order to demonstrate the presence of the sulphocyanides, it is usually only necessary to heat a few cubic centimeters of the pure saliva, faintly acidified with hydrochloric acid, with a dilute solution of ferric chloride, when a red color will be seen to develop. If necessary, larger quantities, such as 100 c.c., are evaporated to a small volume; the test is then applied to the concentrated fluid.

The *test for nitrites* is conducted in the following manner: About 10 c.c. of saliva are treated with a few drops of *Ilasvay's reagent* and heated to a temperature of 80° C., when in the presence of nitrites a red color will develop. The reagent is prepared as follows: 0.5 gram of sulphanilic acid in 150 c.c. of dilute acetic acid is treated with 0.1 gram of naphtylamin dissolved in 20 c.c. of boiling water. After standing for some time the supernatant fluid is poured off and the blue sediment dissolved in 150 c.c. of dilute acetic acid. The solution is kept in a *sealed* bottle.

Of organic matter, ptyalin, a little albumin mixed with mucin, and about 1 gram of urea pro liter are found.

In neutral or slightly alkaline, but not in acid solutions ptyalin rapidly transforms boiled starch into dextrins and sugar at a temperature of from 35° to 40° C.

In order to *test for ptyalin,* a few cubic centimeters of saliva are filtered and added to a solution of starch; the mixture is placed in the warm chamber for 5 to 10 minutes, when it is tested with cupric sulphate or iodine. At first starch gives a blue color with iodine; after digestion has proceeded farther a red or violet red is obtained, indicating the presence of erythrodextrin, while no change in color at all results when achroödextrin only is present. The maltose may be recognized by the fact that it turns the plane of polarization more strongly to the right than glucose; like glucose, it reduces Fehling's solution.

Microscopic Examination of the Saliva.

If normal saliva is allowed to stand, two layers will be seen to form, viz., an upper clear and a lower cloudy layer, which latter contains certain morphological elements. Among these, salivary corpuscles, pavement epithelial cells, and microörganisms are found (Fig. 57).

The salivary corpuscles resemble white corpuscles very closely, but differ in their greater size and coarser appearance. The epithelial cells are large, irregular, polygonal cells, provided with well-defined nuclei and nucleoli; they exhibit certain irregularities in size, according to their origin, and belong to the class of pavement or stratified epithelium.

Microörganisms.[1]—While schizomycetes and molds are only exceptionally found in the mouth under normal conditions, bacteria are always present in large numbers, and it is not surprising that all forms which are found in the air, food, and drink may here be encountered. Some of these, such as the Leptothrix buccalis innominata, Bacillus buccalis maximus, Leptothrix buccalis maxima, Iodococcus vaginatus, Spirillum sputigenum, and Spirocheta dentium, are always present. Together with other bacteria, they have been found in carious teeth, in abscesses communicating with the mouth and pharynx, and in exudates on the mucous membranes of these parts. In all probability, however, they are non-pathogenic. To this class also belongs the smegma bacillus, which has been encountered in the saliva, the coating of the tongue, and in the tartar of the teeth of perfectly healthy individuals. The Leuconostoc hominis also is a normal inhabitant of the oral cavity, but occurs in larger numbers in inflammatory diseases (scarlatina, measles, and diphtheria).[2]

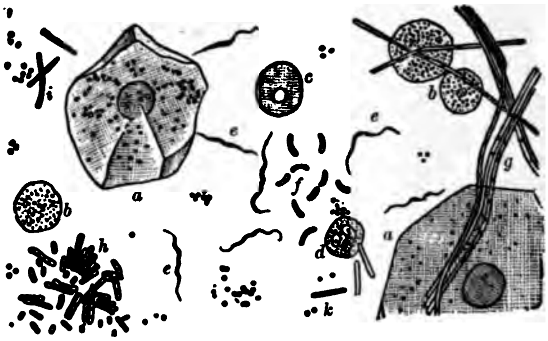

FIG. 57.—Buccal secretion. (Eye-piece III, obj. Reichert, $^1/_{15}$ homogeneous immersion: Abbe's mirror, open condensers.) *a*, epithelial cells; *b*, salivary corpuscles; *c*, fat drops; *d*, leukocytes; *e*, Spirochæta buccalis; *f*, comma bacillus of mouth; *g*, Leptothrix buccalis; *h*, *i*, *k*, various fungi. (v. Jaksch.)

In this connection it is interesting to note that, in contradistinction to the bacteria which are only temporarily found in the mouth, the majority of those which are constantly present cannot be cultivated on artificial media.

Important from a practical standpoint is the fact that a number of pathogenic microörganisms may be found under normal conditions. The Diplococcus pneumoniæ has thus been found in a virulent condition in from 15 to 20 per cent. of healthy individuals, and it is even claimed that in a non-virulent state it is *constantly* present in the mouth. Streptococci are likewise frequently observed, but usually possess but little virulence or none at all when obtained from the healthy mouth and tested upon animals. Pyogenic staphylococci may also be found at times, but are less common than the streptococci. Most important is the occasional occurrence of the diphtheria bacillus in the mouths of individuals who have not been exposed to contagion. Welch[1] mentions that virulent organisms were found by Park and Beebe in the healthy throats of 8 out of 330 persons in New York who gave no history of direct contact with cases of diphtheria; 2 of these 8 persons later developed the disease. Non-virulent bacilli were found in 24 individuals of the same series, and pseudodiphtheria bacilli in 27.

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[1] W. D. Miller, Die Mikroörganismen d. Mundhöhle, 1892.
[2] Hlava, Folia hæmatol., vol. i, p. 612.

Other pathogenic bacteria which may be found in normal mouths are the Micrococcus tetragenus, the Bacillus pneumoniæ of Friedländer, the Bacillus crassus sputigenus, and the Bacillus coli communis.

Pathological Alterations.

It has been mentioned that about 1500 grams of saliva are secreted in the twenty-four hours. This quantity is, however, subject to great variation. An increase is thus frequently noted in pregnancy, in various neurotic conditions, in tabes, bulbar paralysis, in inflammatory diseases of the mouth, in dental caries, following the administration of pilocarpine, in poisoning with mercury, acids, and alkalies, etc. The quantity is diminished in all febrile diseases, in diabetes, and often in nephritis. The effect of psychic influences upon the secretion of saliva as well as of other glands is well known, an increase or decrease in the flow being produced under various conditions.

In determining whether or not salivation actually exists, the physician should not only be guided by the statements of the patient, but an actual estimation of the amount secreted within a definite period of time should be made. Nervous individuals not infrequently complain of "salivation," when a direct estimation will show that the amount is not only not increased, but actually diminished.

An acid reaction has been noted in various diseases of the intestinal tract, in febrile diseases, and notably in diabetes. According to Strauss and Cohn, however, an alkaline reaction is the rule even under pathological conditions.

Among the qualitative changes may be mentioned an increase in the amount of urea, which has been repeatedly observed in nephritis.

Urea may be demonstrated as follows: The saliva is extracted with alcohol, the filtrate evaporated, and the residue dissolved in amyl alcohol. This is allowed to evaporate spontaneously, when crystals of urea will separate out, and may be further examined (see Urine).

Bile-pigment and sugar have not been found in the saliva.

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[1] Dennis' System of Surgery; Surgical Bacteriology.

SPECIAL DISEASES OF THE MOUTH.

Tuberculosis.—In cases of lupus and the so-called benign form of tuberculosis of the mouth it is rarely possible to demonstrate the presence of tubercle bacilli, even in scrapings taken from the base of the ulcers or in the diseased tissue itself, while in cases of ulcerative stomatitis associated with phthisis in its advanced stages they may be frequently found in large numbers. In some cases, however, their demonstration is by no means easy. In the saliva they are only exceptionally seen.

Actinomycosis.—In cases of actinomycosis it is occasionally possible to demonstrate the presence of the specific organism in or about carious teeth. More commonly, however, the patients are not seen until the primary symptoms of the disease have disappeared, when the typical kernels can no longer be found at the *original* points of entry or have become unrecognizable owing to calcification and retrogressive changes.

Usually the disease has already progressed to the formation of a distinct tumor or abscess, and it may then be necessary to make an exploratory incision, and to examine the scrapings which are brought away. The number of kernels which may be found is at times very small, but a careful examination will probably always lead to their detection if the disease in question is actinomycosis.

Catarrhal Stomatitis.—In this affection the quantity of saliva is increased. Microscopically an increased number of epithelial cells and many leukocytes are noted, their number depending upon the intensity of the morbid process.

Ulcerative Stomatitis.—In this condition, following mercurial poisoning or scurvy, the same appearance is noted microscopically as in simple stomatitis. In addition there may be necrotic tissue, red blood corpuscles, and innumerable leukocytes. The reaction of the saliva is intensely alkaline, the color markedly brown, and its odor fetid.

Gonorrheal Stomatitis.—The number of cases of gonorrheal stomatitis that have thus far been recorded is small. The disease, however, has received but little attention, and is probably more common than is generally supposed. In suspected cases the exudate which forms upon the gums, the tongue, and the palate should be examined for gonococci.

Thrush.—Oïdium albicans (Fig. 58) is most commonly seen in children, but may also occur in adults, and especially in phthisical individuals, and sometimes lines the entire mouth. If in such cases a bit of the membrane is pulled off and examined microscopically, it will be found to consist of epithelial cells, leukocytes, and granular detritus, with a network of branching, band-like formations, which present distinct segments. The contents of the segments are clear, and usually contain two highly refractive granules—the spores, one of which is situated at each pole. These segments diminish in size toward the end of each band, their contents at the same time becoming slightly granular.

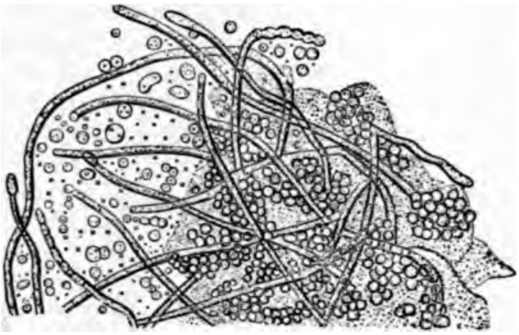

FIG. 58.—Oïdium albicans, the vegetable parasite of thrush. (Reduced from Ch. Robin.)

Tartar.—In a bit of tartar scraped from the teeth actively moving spirochetes are seen, as well as long, usually segmented bacilli, frequently forming bands which are colored bluish red by a solution of iodopotassic iodide. Leptothrix buccalis, shorter bacilli (which are not colored by this reagent), micrococci, and a large number of leukocytes and epithelial cells which have undergone fatty degeneration, are also found. Infusoria have been found by Sternberg, P. Cohnheim, v. Leyden, and others.

COATING OF THE TONGUE.

A brown coating of the tongue is often observed in severe infectious diseases, and consists of remnants of food and incrustated blood. Microscopically, in addition to a large number of epithelial cells, enormous numbers of microörganisms and a large number of dark, cell-like structures, probably derived from desquamated epithelial cells, are found. The white coating of the tongue contains epithelial cells, many microörganisms, and a few salivary corpuscles.

COATING OF THE TONSILS.

Pharyngomycosis Leptothrica.—In the pyoid masses derived from the crypts of the tonsils in cases of follicular tonsillitis, and also in persons who have had frequent attacks of tonsillitis, large numbers of lymphocytes of all sizes are seen, besides epithelial cells and long, segmented fungi—the Leptothrix buccalis (Fig. 59)—which are colored bluish red by a solution of iodopotassic iodide. Ordinary polynuclear neutrophiles are only present in small numbers. At times patches composed of these fungi extend over a considerable area of the tonsils, so that it may be doubtful whether or not the disease is a beginning diphtheria.

More extensive invasions have been described by Dubler, who noted a leptothrix mycosis involving the pharynx, esophagus, and larynx; and by Baginsky in the case of the pharynx, trachea, and nose.

LITERATURE.—Fränkel, Berlin. klin. Woch., 1873, p. 94. Miller, Die Mikroorganismen der Mundhöhle, 1889, Leipzig. Stern, Münch. med. Woch., 1893, p. 381. Hering, Zeit. f. klin. Med., 1884, p. 358. Dubler, Virchow's Arch., 1891, vol. cxxvi, p. 454. Baginsky, cit. by Hering (vide supra).

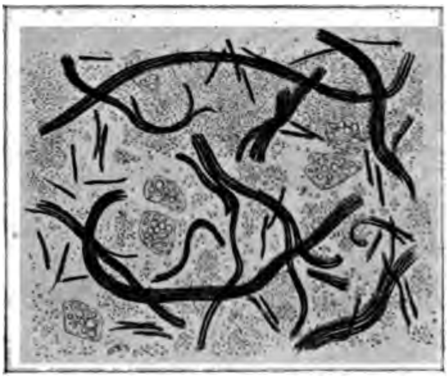

FIG. 59.—Leptothrix buccalis. (v. Jaksch.)

Tonsillitis.—In tonsillitis a large number of bacteria have been isolated from the pseudomembranous deposits. Among the more important which are supposed to bear a causative relation to the disease may be mentioned the various streptococci, staphylococci, less commonly the pneumococcus, the Micrococcus catarrhalis, the Bacillus coli communis, the bacillus of Friedländer, the Bacillus septicæmiæ sputi, and in a few isolated instances the Micrococcus tetragenus. In many cases in which tonsillar deposits are clinically regarded as diphtheritic culture reveals only an abundance of the thrush fungus.

Meyer,[1] in v. Leyden's clinic, succeeded in cultivating a diplostreptococcus from the tonsils in five cases of acute rheumatism with angina, and reports that bouillon cultures of the organism produced characteristic polyarticular arthritis in rabbits. The same organism apparently was also obtained by Allaria[2] in Bozzolo's clinic, and it is interesting to note that his cases resulted from manifest contagion.

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[1] Deutsch. med. Woch., 1901, vol. xxvii, p. 81.

[2] Revista critica di clinica Medica, 1901, vol. ii, p. 805.

Vincent's Angina.—In cases of Vincent's angina (ulceromembranous angina and stomatitis) smears from the exudate will be seen to contain innumerable organisms which are essentially of two types, viz., spirilla and long, fusiform bacilli (Fig. 60). Occasionally, though exceptionally, the bacilli only may be found. The spirilla usually present three or four convolutions and are generally actively motile. They measure from 36 to 40 μ in length by 0.5 μ in breadth. The bacilli measure from 6 to 12 μ in length and are somewhat stouter in the middle than at the ends. They may occur in twos, joined end to end, and usually scattered uniformly throughout the preparation. They are non-motile. Spirilla and bacilli are readily stained with a dilute solution of carbol fuchsin (1 to 20), which should be filtered before use. Löffler's blue and gentian-aniline water may likewise be used.

The bacilli are obligate anaërobes; the spirilla may be obtained together with the bacilli in mixed cultures.

Of late the opinion has been expressed that the spirilla and bacilli may represent stages in the life history of a trypanosome.

Both organisms have occasionally been found associated with diphtheria bacilli.

The disease seems to be more common than was first thought. The earlier cases were reported by Vincent, Bernheim, Conrad, and others. In the United States the disease has been described by Mayer, Fisher, Crandall, Weaver and Tunnicliff, Berkeley, and others.

LITERATURE.—J. W. Byers, Lancet and Brit. Med. Journ., January 9, 1904. Weaver and Tunnicliff, Journ. of Infect. Dis., 1905 vol. ii, p. 446. Berkeley, Med. News, 1905, vol. xxxvi, p. 976. Wright, 1904, July 4, p. 73.

Diphtheria.—Recognizing the great importance of an early diagnosis in cases of diphtheria, an examination for Löffler's bacillus has become just as important today as that for the bacillus of tuberculosis.

By means of a stout platinum loop, a pair of forceps, or a cotton swab, a piece of membrane is scraped from the tonsils, the soft palate, or the pharynx. From this cultures are prepared as described below; at the same time smears are made on slides and fixed, when air dry, by being passed several times through the flame of a Bunsen burner. They are then stained for five to ten minutes in Löffler's alkaline solution of methylene blue, which consists of 30 c.c. of a concentrated alcoholic solution of methylene blue in 100 c.c. of an aqueous solution of potassium hydrate (1 to 10,000). They are then rinsed in water, dried and examined with a $\frac{1}{12}$ oil-immersion lens.

A rapid method of staining, and one which also gives satisfactory results, is suggested by Neisser. The organism is grown on ox-blood serum and examined after nine to twenty-four hours. The air-dried smears are placed for one to three seconds in a solution composed of 20 c.c. of an alcoholic solution of methylene blue (1 to 20 c.c. of 90 per cent. alcohol), 950 c.c. of distilled water, and 30 c.c. of glacial acetic acid. They are then washed in water, stained for three to five seconds in a 0.2 per cent. hot and filtered aqueous solution of vesuvin, again washed off, dried in the air, and mounted in balsam. The bacilli are brown and have in their interior 2 to 4 blue granules which are usually located near the poles.

The following method also may be employed, as suggested by Schauffler. The staining reagent has the following composition:

Filtered solution of Löffler's methylene blue	10.0 c.c.
Filtered solution of pyronin (0.5 gram to 10 c.c. of water) .	1.5 c.c.
Acid alcohol (3 c.c. of 25 per cent hydrochloric acid to 97 c.c. of absolute alcohol)	0.5 c.c.

Cover-glass specimens are stained for one minute; they are then washed in running water and mounted in balsam as usual. The bacilli are stained blue, the pole bodies a bright ruby red.

Pseudodiphtheritic bacilli are said to take only the blue stain with this method.

The organism grows best on Löfflers blood serum; upon this it develops so much more rapidly than other organisms which are usually present in the secretions of the mouth and throat, that, after six to eight hours' incubation at 34° to 35° C., it often forms the only colonies that attract attention. Smears are then made and stained according to Neisser's or Löffler's method.

In the absence of blood serum, bouillon, nutrient gelatin, nutrient agar, glycerin agar, and potato may be employed. Coagulated egg albumen, as pointed out by Booker, and milk are also good media. But it is to be noted that the "typical" staining effect with Neisser's method is commonly only obtained if the organism has been grown on ox-blood serum, and if the growth is not older than twenty-four hours.

According to Knapp the true bacilli, in contradistinction to the pseudodiphtheria bacilli, will ferment dextrose and maltose. The Bacillus xerosis will do the same. In contradistinction to the diphtheria organism the Bacillus xerosis will ferment cane sugar; the former, in contradistinction to the xerosis, will ferment dextrin. The fermentation tests must be made with the litmus serum-water media of His.[1] Results after twenty-four hours' growth at 37° C.: Pseudodiphtheria—none of the sugars fermented; media remain blue. Diphtheria—dextrose, mannite, maltose, and dextrin fermented; media red and coagulated. Saccharose not fermented. Xerosis bacillus—dextrose, mannite, maltose, and saccharose fermented with acid production; media red and coagulated. Dextrin not fermented. The Bacillus xerosis, moreover, forms a very thin scum or pellicle on the surface of the media which is absent with the other bacteria.

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[1] Journ. of Med. Res., vol. xii, p. 475–478. See also Appendix: Media.

The colonies are large, round, elevated, and grayish white in color, with a centre that is more opaque than the slightly irregular periphery. The surface of the colony is at first moist, but after a day or two it assumes a dry appearance.

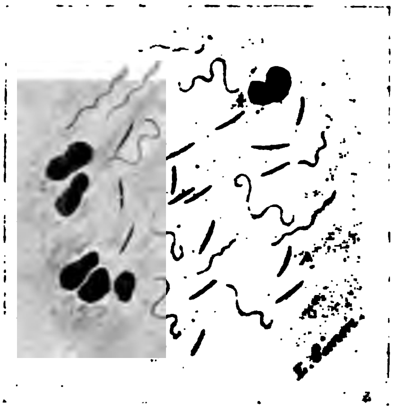

FIG. 60.

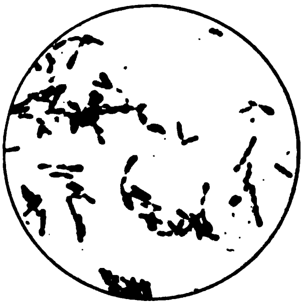

FIG. 61.

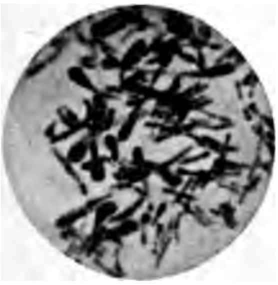

FIG. 62.

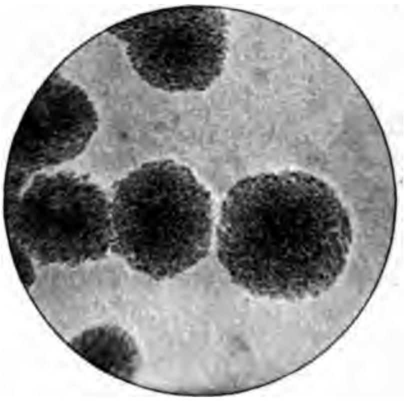

FIG. 63.

FIG. 60.—Spirilla and fusiform bacilli of Vincent's angina.
FIG. 61.—Characteristic forms of diphtheria bacilli from blood-serum cultures, showing clubbed ends and irregular stain. × 1100 diameters. (Park.)
FIG. 62.—B. diphtheriæ. Forty-eight hours' agar culture. Thick, medium-clubbed rods and moderate number of segments. One year on artificial culture media. × 1410 diameters. (Park.)
FIG. 63.—Colonies of diphtheria bacilli. × 200 diameters. (Park.)

The bacillus (Figs. 61, 62, and 63) is non-motile and varies in size and shape, its average length being from 2.5 to 3 μ, its breadth from 0.5 to 0.8 μ. Its morphological characteristics are so peculiar as to render its identification upon cover-slip preparations and in sections of the diphtheritic membrane an easy matter in most cases.

Sometimes the organism appears as a straight or slightly curved rod; but especially characteristic are irregular and often bizarre forms, such as rods with one or both ends terminating in little bulbs and rods apparently broken at intervals, in which short, well-defined, round, oval, or straight segments can be made out. Very commonly two organisms lie together forming an obtuse angle, or numbers of them may be observed lying side by side.

Some forms stain uniformly, others in an irregular manner; the most typical appearance is that of little granules near the poles of the bacillus, which stain blue with Neisser's method, while the body of the organism is colored brown.

Streptococci are also seen as a rule, and it may be said that the gravity of a case is directly proportionate to the number of streptococci present.

It is important to note that diphtheria bacilli may still be found in the throat for weeks after all clinical symptoms have disappeared. Patients should hence be isolated until a bacteriological examination has demonstrated the absence of the organism.

LITERATURE.—S. Flexner, "The Bacteriology and Pathology of Diphtheria," Johns Hopkins Hosp. Bull., 1895, p. 39. W. H. Welch, Amer. Jour. Med. Sci., 1894. Heubner, Schmidt's Jahrbücher d. gesammten Med., 1892, vol. ccxxxvi, p. 270. Klebs, Arch. f. exper. Path., 1875, vol. iv, p. 207. Löffler, Centralbl. f. Bakt. u. Parasit., 1887, vol. ii, p. 105: and 1890, vol. vii, p. 528. C. Fränkel, "Die Unterscheidung d. echten u. d. falschen Diphtheriebacillen," Berlin. klin. Woch., 1897, p. 1087. W. G. Schauffler, Med. Record, December 6, 1902.

Scarlatina.—According to Baginsky, streptococci are practically constantly found in the pharyngeal secretion.

LITERATURE.—A. Baginsky, Deutsch. med. Woch., October 23, 1902.

Glandular Fever.—According to Neumann and Comby, glandular fever generally depends upon infection with a streptococcus. In the case reported by Lande and Froin and by Hirtz[1] bacteriological examination of the throat at the height of the febrile stage revealed the presence of the pneumococcus in a virulent condition.

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[1] Lande et Froin, Rev. mensuelle des Mal. de l'Enfance, 1901, p. 78.

CHAPTER III.

THE GASTRIC JUICE AND GASTRIC CONTENTS.

THE SECRETION OF GASTRIC JUICE.

THE gastric juice is the result of the glandular activity of the stomach, and is the only secretion of the digestive tract which presents an acid reaction.

As is well known, the mucous membrane of the stomach is covered throughout its entire extent by a single layer of cylindrical epithelium, which dips down in places to line the orifices and larger ducts of the numerous tubular glands with which it is beset. Of these, two kinds are described, viz., the fundus and pyloric glands, so named from the location in which they are principally found. In the secretory portion of a fundus gland two sets of cells can be distinguished. The one kind is small, granular, and polyhedral or columnar, bordering upon the narrow lumen of the tube: these are termed the chief or principal cells (Heidenhain), but are also known as the central or adelomorphous cells. They stain with aniline dyes to only a slight extent. The others, known as parietal, adelomorphous, or oxyntic cells, are variously situated between the adelomorphous cells and the membrana propria; they are most numerous in the necks of the glands. They are larger than the chief cells, oval or angular and finely granular in structure; they possess a strong affinity for the aniline dyes. The pyloric glands, which are found only in the region of the pylorus, on the other hand, are characterized by the greater length of their ducts, which are also lined by the cylindrical epithelium of the mucous membrane proper. The secretory portion of these glands is represented by a single layer of short and finely granular, columnar cells, which closely resemble the chief cells of the fundus glands. In addition to these, a few isolated cells, the cells of Nussbaum, are found, which in structure and in their behavior to aniline dyes resemble the parietal cells.

Upon chemical examination the gastric juice is found to consist essentially of water, free hydrochloric acid, pepsin, rennet (a milk-curdling ferment), lipase, mucus, and certain mineral salts.

Of these constituents hydrochloric acid is secreted by the parietal cells, pepsin, the milk-curdling ferment, and lipase by the chief cells of the fundus and the pyloric glands, while the mucus is the product of the cylindrical goblet-cells lining the stomach and the wider portions of its glandular ducts. It should be borne in mind that the ferments do not exist in the cells as such, but as zymogens, which are transformed into the ferments through the activity of the free hydrochloric acid. According to modern investigations, moreover, the zymogens only are *secreted* by the cells.

Until recently it was supposed that the gastric juice is secreted only upon appropriate stimulation of the nervous mechanism of the stomach, either directly or indirectly, and that the stomach in its quiescent state—*i. e.*, when not digesting—is empty. The researches of Schreiber and Martius, however, have rendered the correctness of this view doubtful, as they were able to obtain quantities of gastric juice, varying from 1 to 60 c.c., from the non-digesting stomach of every normal person examined.

Test Meals.—As the amount of hydrochloric acid which is secreted varies with the amount and the character of the food ingested, it has been found useful for purposes of comparison to make analyses after the administration of test meals of constant composition. The most important test meals are the following:

The Test Breakfast of Ewald and Boas.—This consists of 35 grams of wheat bread and 400 c.c. of water or weak tea, without sugar. It is best to give this meal to the patient early in the morning, when the stomach is empty—*i. e.*, as a breakfast, and in cases of dilatation or of marked atony, after previous lavage. The gastric contents are obtained one hour later.

The Test Breakfast of Boas.—This consists of a plateful of oatmeal soup, prepared by boiling down to 500 c.c. one liter of water to which one tablespoonful of rolled oats has been added. A little salt may be used if desired, but nothing more. The contents of the stomach are obtained one hour later. This test meal was devised by Boas in order to guard against the introduction from without of lactic acid, which is present in all kinds of bread. The meal is employed in cases of suspected cancer of the stomach in which a quantitative estimation of lactic acid is to be made, the stomach being washed out completely the night before.

The Test Dinner of Riegel.—This consists of a plate of soup (400 c.c.), a beefsteak (150 to 200 grams), and 150 grams of mashed potatoes. The contents of the stomach are obtained after four hours. The disadvantage of this method lies in the fact that the lumen of the stomach tube is frequently occluded by pieces of undigested meat, a source of annoyance which may be guarded against by using finely chopped meat. Moreover, a positive lactic acid reaction (referable to sarcolactic acid) is obtained in a large number of cases, and entirely irrespective of the amount of hydrochloric acid present.

The Double Test Meal of Salzer.—For breakfast the patient receives 30 grams of lean, cold roast, hashed or cut into strips sufficiently small not to obstruct the stomach tube; 250 c.c. of milk; 60 grams of rice, and 1 soft-boiled egg. Exactly four hours later the second meal is taken, consisting of 35 to 70 grams of stale wheat bread and 300 to 400 c.c. of water. The gastric contents are withdrawn one hour later. In this manner the gastric juice is not only obtained at the height of digestion, but an idea may at the same time be formed of the motor power of the stomach. Under normal conditions the organ should contain no remnants of the first meal at the time of examination.

The Stomach Tube.—The stomach tubes in general use are essentially large Nélaton catheters. They should measure from 72 to 75 cm. in length, and be provided with three fenestra, of which one is placed at the end of the tube and two laterally, as near the end as possible. For the purpose of washing out the stomach the tube is connected with a glass funnel.

It is important that the tubes should be thoroughly cleansed in hot water as soon after use as possible. The advice of Boas, moreover, to have special marked tubes for tuberculous, syphilitic, and carcinomatous patients should be borne in mind. Patients in whom lavage is to be practised for any length of time should provide their own instruments.

Contra-indications to the Use of the Tube.—Of direct contra-indications to the use of the tube there should be mentioned the existence of the various forms of valvular disease when in a state of imperfect compensation, angina pectoris, arteriosclerosis of high degree, aneurysm of the large arteries, recent hemorrhages from whatever cause, marked emphysema with intense bronchitis, acute febrile diseases, etc.

Introduction of the Tube.—The technique of the introduction of the tube should be as simple as possible; the exhibition of complicated bottle arrangements for the purpose of obtaining the gastric juice only adds to the excitement of a nervous patient, and should be avoided. The patient's clothing and floor of the room should be protected from being soiled by material that may be vomited along the sides of the tube, the dribbling of saliva, etc. For this purpose, Türk's rubber bib[1] with pouch may be advantageously employed.

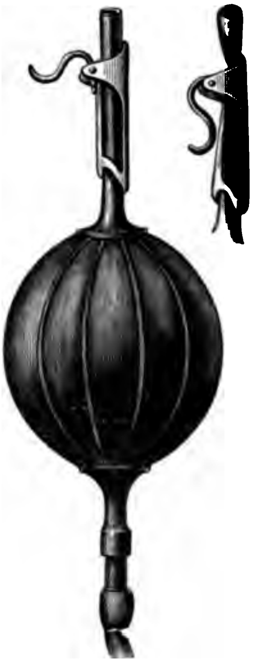

FIG. 64.—Boas' bulbed tube.

Cocainization of the pharynx is not necessary, but may be resorted to in hyperesthetic individuals, a 10 per cent. solution being employed.

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[1] Manufactured by G. Tiemann & Co., New York.

The tube, held like a pen, is passed to the posterior wall of the pharynx, the patient bending his head *forward, and not backward,* as is usually advised. The patient is then told to swallow. The tube is pushed until resistance is felt when it meets with the floor of the stomach. At the least sign of cyanosis or of marked pallor the tube should be withdrawn at once, and the patient observed for a day or two before a second attempt is made.

If the gastric juice does not flow at once, the patient is instructed to bear down with his abdominal muscles, and, if this is insufficient, to cough a little. Repeated attempts of this kind will usually bring about the desired result, unless the tube has not been introduced far enough or too far; in the latter case it will double upon itself, so that its end rises above the level of the liquid. Pressing upon the abdomen with the hands is of no effect (Method of Expression).

Aspiration must at times be employed. For this purpose Boas' bulbed tube (Fig. 64) is convenient. The manner in which it is used is the following: The proximal end of the tube, after having been introduced into the stomach, is compressed and the bulb squeezed, when the distal end is clamped and the bulb allowed to expand. A partial vacuum is thus produced, which usually has the desired effect. In the absence of such an instrument the stomach tube may be connected with a bottle, in which a partial vacuum has been established by aspiration (Fig. 65). Unless the patient is accustomed to the introduction of the tube, however, these more complicated procedures should be avoided as much as possible (Method of Aspiration).

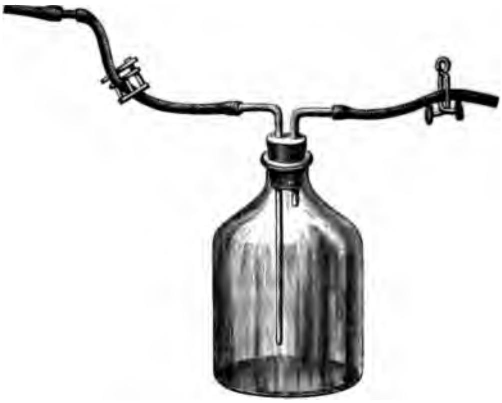

FIG. 65. - Arrangement of a bottle for aspiration of the gastric contents.

In order to *wash out the stomach*, the funnel is filled with lukewarm water or any desired medicated solution, elevated above the head of the patient, and the water allowed to flow. From 500 to 1000 c.c. may be introduced at one time. By depressing and inverting the funnel over a suitable vessel before all water has left the funnel a siphon arrangement is established and the stomach emptied. It is well to measure the returning water as well as the amount introduced. Should the flow diminish or cease before all the water has been removed, the end of the tube probably stands above the level of the liquid, and the flow can be started again by pushing the tube on farther or by withdrawing it a little, as the case may be.

Washing out the stomach soon after the ingestion of a full meal is always very tedious and annoying, if not an impossible procedure, as the fenestra readily become obstructed. Should this occur, the funnel, filled with water, is elevated as high as possible, with a view to overcome the obstruction by hydrostatic pressure; or, if this proves insufficient, the funnel is detached and the obstruction is lodged by means of air, for which purpose a Politzer bag or the bulb of a Boas tube is very convenient.

GENERAL CHARACTERISTICS OF THE GASTRIC JUICE.

Pure gastric juice is an almost clear, faintly yellowish fluid, of a sour taste and a peculiar characteristic odor. Its specific gravity varies between 1.002 and 1.003, corresponding to about 0.5 per cent. of solids. Its reaction, owing to the presence of hydrochloric acid, is acid.

Amount.—Very little is known of the total quantity of gastric juice that is secreted in the twenty-four hours. The figure given by Beaumont,[1] viz., 180 grams pro die, based upon observations made upon the often-quoted Canadian hunter, Alexis St. Martin, is undoubtedly too low. The amount given by Bidder and Schmidt,[2] viz., that corresponding to about one-tenth of the body weight, is probably more nearly correct.[3] It may be stated *a priori* that the quantity secreted varies within wide limits, being influenced by numerous factors, notably by the degree of the appetite and the amount and character of the food taken, especially that of the proteids. The age and sex of the individual, the time of day (notably in its relation to the ingestion of food), the emotions, etc., all influence the glandular activity of the stomach.[4]

From the non-digesting organ from 1 to 60 c.c. of gastric juice may be obtained at one time. The amount which can be procured during the process of digestion, on the other hand, varies with the amount of liquid ingested, the time of expression, the size and motor power of the stomach, and the degree of transudation; the process of resorption probably does not play any part, as it has been ascertained that very little water, if any, is absorbed in the stomach.

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[1] Experiments and Observations on the Gastric Juice, Boston, 1834.

[2] Verdauungssäfte u. d. Stoffwechsel, 1852.

[3] Grünewald's figure—*i. e.*, 1580 grams—I likewise regard as too low. According to my experience, the daily secretion appears to vary between 2000 and 3000 c.c.

[4] See C. E. Simon, Physiological Chemistry, third edition, 1907, Lea Bros. & Co.

As a rule from 20 to 50 c.c. of filtrate can normally be obtained one hour after the ingestion of Ewald's test breakfast.

Abnormally large quantities of gastric juice are practically found only in cases of so-called *hypersecretion*, the "Magensaftfluss" of the Germans, which may occur periodically or continuously. Formerly the presence of appreciable quantities of gastric juice in the non-digesting organ was regarded as conclusive evidence of the existence of this condition, but in the light of Schreiber's researches this position can no longer be maintained. The diagnosis should, hence only be made when in conjunction with the clinical symptoms of hypersecretion from 100 to 1000 c.c. of pure *gastric juice* can be obtained from the non-digesting organ. To this end, the stomach should be emptied completely by the tube before retiring, and an examination made on the following morning, no foods or liquids being allowed in the mean time.

In various pathological conditions abnormally large quantities of liquid may be obtained, which cannot be regarded as gastric juice, however. Attention will be drawn to these conditions at another place.

CHEMICAL EXAMINATION OF THE GASTRIC JUICE.

The Acidity of the Gastric Juice is Referable to the Presence of Free Hydrochloric Acid.—It has been conclusively demonstrated by Schmidt that the acidity of the gastric juice is due to the presence of free hydrochloric acid and to this only. After accurately determining the amount of chlorine and all basic substances present, it was found that after the latter had been saturated a quantity of hydrochloric acid still remained, which in the dog varied between 0.25 and 0.42 per cent., with an average of 0.33 per cent. The amount of free acid was also determined by titration and the same results reached as by gravimetric analysis.

While it can thus be regarded as an established fact that hydrochloric acid only is found in the gastric juice, such as it is secreted, there can be no doubt that traces of lactic acid may be found in the stomach contents during the process of digestion. These traces, however, have been introduced from without.

The time at which hydrochloric acid will appear in the free state depends *cæteris paribus* upon the quantity of albumins ingested. With Ewald's test breakfast it appears after thirty-five minutes and reaches its maximum between fifty and sixty minutes after eating. With Riegel's meal the time is longer; it appears after one hundred and twenty to one hundred and fifty minutes in the free state and reaches its maximum after one hundred and eighty to two hundred and ten minutes.

Under pathological conditions the amount of free hydrochloric acid, as will be shown, may undergo great variations, diminishing on the one hand to zero, and increasing on the other to 0.5 per cent., or even more. In other cases lactic acid and other organic acids may appear in notable amounts.

Method of Determining the Total Acidity of the Gastric Contents.—To this end a known quantity of gastric juice is titrated with a one-tenth normal solution of sodium hydrate, using phenolphthalein as an indicator, when the number of cubic centimeters of the one-tenth normal solution employed, multiplied by the equivalent of 1 c.c. of this solution in terms of hydrochloric acid, will indicate the amount of acid present, from which the percentage acidity is readily calculated.

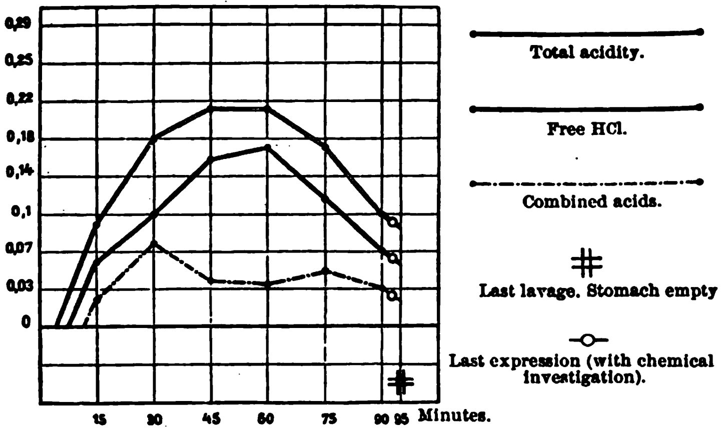

FIG. 66.—Course of the acidity of the gastric juice after a test meal of 300 grams of tea and 50 grams of bread. (Schüle.)

Method.—5 or 10 c.c. of filtered gastric juice are titrated with the one-tenth normal solution of sodium hydrate, using 2 or 3 drops of a 1 per cent. alcoholic solution of phenolphthalein as an indicator until a permanent rose color appears. The number of cubic centimeters of the one-tenth normal solution employed multiplied by 0.00365 will indicate the acidity of the 5 or 10 c.c. of gastric juice in terms of HCl, from which the percentage acidity is calculated.

Example.—10 c.c. of gastric juice required the addition of 6.5 c.c. of the one-tenth normal solution; 6.5 × 0.00365 (*i. e.*, 0.0237) would hence indicate the acidity of the 10 c.c. of gastric juice in terms of HCl, and 0.0237 × 10 = 0.237, the percentage acidity.

Or the result may be expressed in terms of the number of c.c. of the $\frac{n}{10}$ solution which would be necessary to neutralize 100 c.c. of stomach contents. In the example the total acidity would thus be $6.5 \times 10 = 65$. This method of indicating results is indeed the usual.

Under normal conditions figures varying from 40 to 60 are usually obtained one hour after the ingestion of Ewald's test breakfast, while in pathological conditions greater variations are observed. In acute and chronic inflammatory conditions of the stomach, as well as in some of the neuroses, the acidity of the gastric contents is below normal. Higher figures are met with in some cases of ulcer and in some cases of dilatation, but are especially common in neurotic conditions; a degree of acidity corresponding to 90 or even more is then not infrequently observed. Increased acidity, usually associated with hypersecretion of gastric juice, is met with in the so-called *hypersecretio acida et continua* of Reichmann.

Preparation of decinormal alkali solution.—A normal solution of sodium hydrate is one containing the equivalent of its molecular weight in grams—*i. e.*, 40 grams—in 1000 c.c. of distilled water; a decinormal solution will, therefore, contain 4 grams in the same volume of water. This quantity is dissolved in about 900 c.c. and the solution brought to the proper strength by titrating it with a solution of oxalic acid of known strength.

From the equation

$$2NaOH + C_2H_2O_4 = C_2Na_2O_4 + 2H_2O,$$

it is seen that 2 molecules of NaOH (molecular weight 40) combine with 1 molecule of $C_2H_2O_4 + 2H_2O$ (molecular weight 126), or 4 parts by weight of the former with 6.3 of the latter. 6.3 grams of chemically pure, crystalline oxalic acid (which is stable and non-deliquescent) are dissolved in 1000 c.c. of distilled water; this makes a $\frac{n}{10}$ normal solution of the acid. Were the alkali solution of the proper strength it should take just 10 c.c. to neutralize 10 c.c. of the acid. But as the alkali solution cannot be made up accurately from the start (owing to inconstant weight from deliquescence), and as it has been purposely made too strong, less than 10 c.c. will be required, *e. g.*, 8 c.c. It is then ascertained how many such portions of alkali solution there are left, and then a corresponding amount of water is added, *i. e.*, an amount representing the deficit found as compared with the acid solution.

In the present example, for instance, we started with 900 c.c. of the uncorrected alkali solution, of which 8 c.c. were used in the test titration. There are remaining then 892 c.c. For every 8 c.c. in this bulk, viz., 111.5 portions, 2 c.c. of distilled water must be added; hence $111.5 \cdot 2 = 223$ c.c. A second titration is made to ensure the correctness of the result.

Since 1000 c.c. of the one-tenth normal solution containing 4 grams of NaOH are equivalent to 3.65 grams of HCl, as is seen from the equation

$$\begin{array}{llll} \mathrm{NaOH} & +\mathrm{HCl} & = \mathrm{NaCl} & + \mathrm{H_2O} \\ 40 & 36.5 & & \end{array}$$

1000 c.c. of the $\frac{1}{10}$ normal solution represent 3.65 grams of HCl
100 " " " " " " 0.365 gram " "
10 " " " " " " 0.0365 " " "
1 " " " " " represents 0.00365 " " "

It has been pointed out that the reaction of normal gastric juice is always acid, owing to the presence of free hydrochloric acid, and the same may be said to hold good for the gastric contents in general obtained from normal individuals. Pathologically an acid reaction is also the rule, as in those cases in which hydrochloric acid is absent fatty acids and lactic acid usually make their appearance. It is, therefore, not surprising that an alkaline, neutral, or amphoteric reaction is but rarely, or at least not commonly, observed in the gastric contents artificially obtained, and practically seen only in the so-called mucous form of chronic gastritis, or in those rare cases of anadeny, in which a complete destruction of the gastric glands has taken place. In vomited material, on the other hand, such observations are common, owing to the presence of large amounts of saliva. The vomited material in cases of so-called *vomitus matutinus*, which is usually referable to a chronic catarrhal condition of the pharynx, generally presents an alkaline reaction, owing to the fact that the fluid brought up is largely unchanged saliva.

The Amount of Free Hydrochloric Acid.—Pure gastric juice, according to Ewald,[1] Szabó,[2] and Boas,[3] contains from 2 to 3 pro mille of free hydrochloric acid.

In the digesting organ such amounts are met with only at the height of digestion, and after all basic affinities have been saturated. The time at which free hydrochloric acid can be demonstrated in the gastric contents after the ingestion of a meal will, hence, vary with the character of the food and its amount. When but little work is to be accomplished free hydrochloric acid is found much sooner than otherwise. After Ewald's test breakfast, it appears in thirty-five minutes; the point of maximum acidity is reached after from fifty to sixty minutes, and corresponds to 1.7 pro mille. Following Riegel's meal, on the other hand, the free acid appears after one hundred and thirty-five minutes, and reaches its highest point (corresponding to 2.7 pro mille) in from one hundred and eighty to two hundred and ten minutes.

Clinically it is necessary to distinguish between euchlorhydria, or the secretion of a normal amount of free hydrochloric acid (0.1 to

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[1] Loc. cit.

[2] Zeit. f. physiol. Chem., 1877, vol. i, p. 155.

[3] Loc. cit. See also A. Schüle, Zeit. f. klin. Med., 1896, vols. xxviii and xxix.

0.2 per cent.), hypochlorhydria, or the secretion of a deficient amount (less than 0.1 per cent.), hyperchlorhydria, in which more than 0.2 per cent. is found, and anachlorhydria, in which no hydrochloric acid at all is secreted.

Euchlorhydria.—Euchlorhydria, when associated with clinical symptoms pointing to gastric derangement, is most commonly observed in gastric neuroses. A chronic gastritis can always be excluded in the presence of a normal amount of free acid. It may be associated with a certain degree of atony. It was formerly thought that a normal amount of acid would preclude the diagnosis of ulcer, but it is known that this association is quite possible. The same is seen in pyloric stenosis due to a healed ulcer.

Hypochlorhydria.—Hypochlorhydria is associated with all those diseases in which the secretory elements have been more or less damaged, as the result of general disease (anemia, chronic heart and renal lesions, phthisis, chronic icterus, many febrile diseases), or of local disease, as in subacute and chronic gastritis, in some cases of ulcer of the stomach or the duodenum, in incipient carcinoma, and in certain cases of dilatation and atony. The withdrawal of chlorides from the food will also lead to a diminished production of hydrochloric acid.

Anachlorhydria.—Not many years ago it was thought that the absence of free hydrochloric acid was pathognomonic of carcinoma of the stomach. This view was soon abandoned, however, as it was shown that cases of carcinoma occur in which hydrochloric acid is not only present, but present in excessive amounts. This is true especially of those cases in which the malignant growth has started upon the base of an old ulcer. It is noteworthy, moreover, that in early cases of carcinoma, even in the absence of ulcer, hydrochloric acid may at times be demonstrable and then disappear for days and weeks. It was furthermore shown that anachlorhydria exists in almost all cases of advanced chronic gastritis, in pernicious anemia (gastric anadeny), and is a fairly common occurrence in neurasthenic and hysterical individuals. In these cases periods of ana- hyper- and hypochlorhydria may alternate apparently without cause. In the acute febrile infections also anachlorhydria is not uncommon.

Hyperchlorhydria.—Hyperchlorhydria (acid stomach, gastroxynsis) is very common in neurotic individuals, where it may alternate with hypo- and anachlorhydria. The same is seen even normally during menstruation. Associated with a continuous hypersecretion of gastric juice, it constitutes the neurosis known as *hypersecretio acida et continua* (gastrosuccorrhœa acida). Hyperchlorhydria is also of frequent occurrence in cases of gastric ulcer, and may even occur in carcinoma, notably in those cases in which, as stated above, the new-growth has started from an old ulcer. Regarding the frequency of hyperchlorhydria in ulcus there can be no doubt that this is found

PLATE XII.

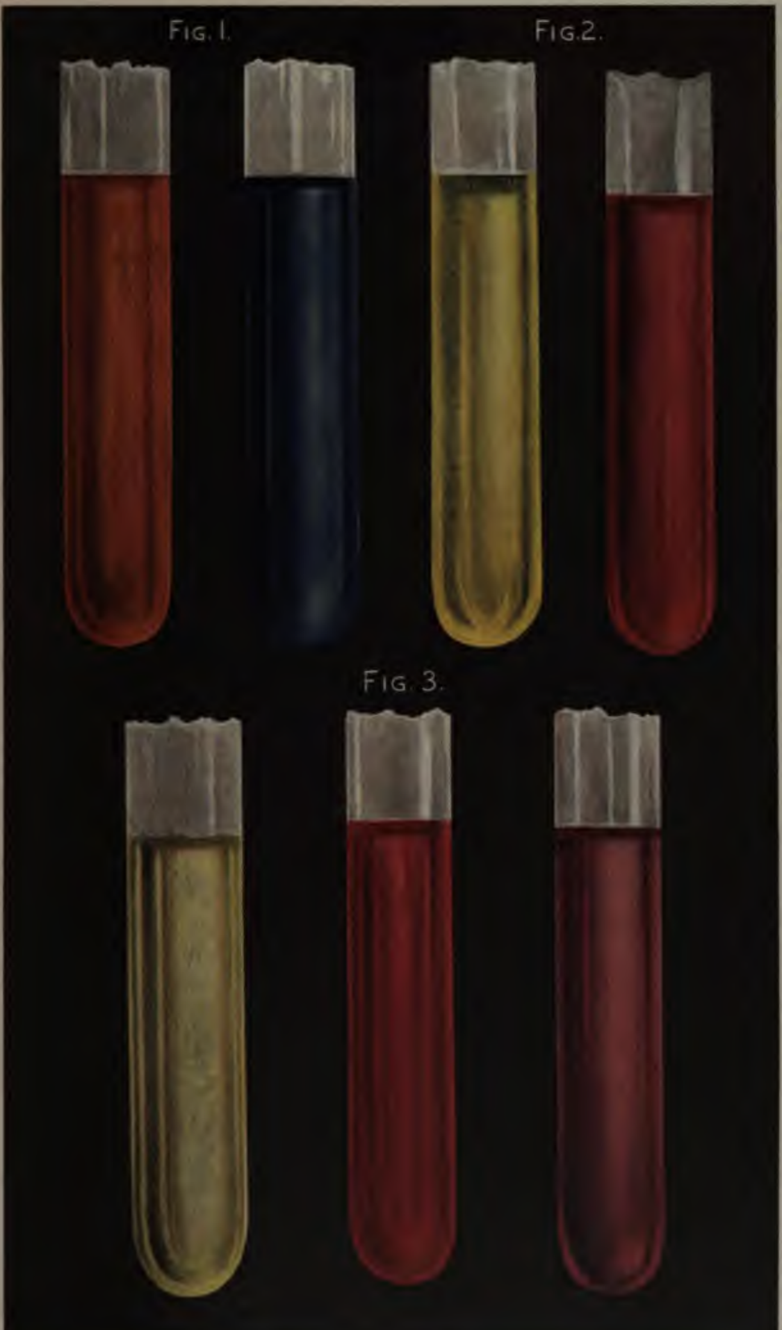
Fig. 1.
Fig. 2.
Fig. 3.

Fig. 1.—Congo-red Test.
Fig. 2.—Dimethyl Reaction.
Fig. 3.—Alizarin Reaction.

in the majority of cases. Normal values, however, are by no means uncommon, and in some instances the amount of hydrochloric acid may be diminished.

Hyperchlorhydria is also met with in passive congestion of the stomach (Schreiber's so-called "stagnant stomach"), in certain types of mental disease, in the early stages of chronic gastritis, during migraine attacks, etc.

Test for Free Acids. **The Congo-red Test.**[1]—Congo-red is a carmine-colored powder, while its solutions are of a peach- or brownish-red color, which changes to blue upon the addition of a free acid, but remains unaffected in the presence of an acid salt. Congo-red may be employed in solution or in the form of a test paper. The latter is less delicate than the solution, and indicates only the presence of 0.01 per cent. of hydrochloric acid, while a positive reaction can still be obtained with the aqueous solution in the presence of 0.0009 per cent. The solution should be moderately dilute. The test paper is prepared by soaking filter paper, free from ash, in this solution, drying, and cutting it into suitable strips. In order to test for free acid, it is only necessary to immerse a strip of the test paper in the filtered gastric juice, or to add a drop or two of the solution to a small amount of the juice, when in the presence of a free acid a blue color will develop, which varies from sky-blue to a deep azure according to the amount present. (Plate XII, Fig. 1.) If the result is positive, the nature of the free acid must be ascertained, and it is, therefore, necessary to test for free hydrochloric acid, or in its absence for lactic acid and certain fatty acids.

Tests for Free Hydrochloric Acid.—The various reagents which may be employed are given below, and are arranged according to their degree of delicacy, viz.:

1. Dimethyl-amido-azo-benzol	0.02 pro mille.
2. Phloroglucin-vanillin	0.05 "
3. Resorcin	0.05 "
4. Tropæolin 00	0.30 "
5. Mohr's reagent	1.00 "

The Dimethyl-amido-azo-benzol Test.[2]—This test has largely replaced the older phloroglucin-vanillin and resorcin tests in the routine work of the clinical laboratory. The delicacy of the reagent is such that the natural yellow color of the indicator is changed to a reddish tinge upon the addition of but 1 drop of a one-tenth normal solution of hydrochloric acid in 5 c.c. of distilled water. Its superior delicacy, as compared with the phloroglucin-vanillin and resorcin tests, is apparent from the fact that 5 c.c. of a 0.5 per cent. solution of egg albumen, to which 6 drops of a one-tenth normal solution of hydrochloric acid have been added, still give a positive reaction with dimethyl-amido-azo-benzol, while the phloroglucin-vanillin and resorcin reactions are negative. Organic acids, including lactic acid, yield a red color only when present in amounts exceeding 0.5 per cent. I have further ascertained that *if albumoses are present, a cherry-red color is not obtained even though lactic acid be present to the extent of* 1 *per cent.* Loosely combined hydrochloric acid and salts do not produce a red color.

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[1] Riegel, Deutsch. med. Woch., 1886, No. 35; and Boas, Diagnostik u. Therapie d. Magenkrankheiten.

[2] Töpfer, Zeit. f. physiol. Chem., 1894, vol. xix. Hari, Arch. f. Verdauungskrank., vol. ii, pp. 182 and 332.

For practical purposes a 0.5 per cent. alcoholic solution is employed; 1 or 2 drops of this are added to a small quantity of the filtered gastric contents; in the presence of free hydrochloric acid a beautiful cherry red develops at once, which varies in intensity with the amount of free acid present (Plate XII, Fig 2.) In the presence of organic acids an orange color is obtained. In watery solution the color is a greenish yellow and the fluid is distinctly fluorescent.

I have used Töpfer's test for many years and am well satisfied with the results. In teaching students it is well to show the color which one obtains with lactic acid in the presence of albumoses; confusion as to whether or not free hydrochloric acid is present will then not occur.

The Phloroglucin-vanillin Test.[1]—The solution employed contains 2 grams of phloroglucin and 1 gram of vanillin, dissolved in 30 c.c. of absolute alcohol; a yellow color results, which gradually turns a dark golden red, changing to brown when exposed to light. The solution should therefore be kept in a dark-colored bottle. Lenhartz suggests the use of separate solutions of phloroglucin and vanillin, 1 or 2 drops of each being employed in the test. Boas recommends a solution of the phloroglucin and vanillin, in the proportions indicated in 100 grams of 80 per cent. alcohol, and claims that the reagent is then still more sensitive and more stable. If a few drops of gastric juice, or even of the unfiltered gastric contents, containing 0.05 per cent. or more of free hydrochloric acid, are treated with the same number of drops of the reagent, no change in color results, but upon slow evaporation—*boiling and rapid evaporation are to be avoided*—a general rose tint or fine rose-colored lines develop, which are characteristic of the presence af the free acid.

For practical purposes it is best to carry on this slow evaporation on a thin porcelain butter dish, the porcelain cover of a crucible, or in a small evaporating dish of the same material. The color obtained in the presence of free hydrochloric acid is a rose color in every instance, and varies in intensity with the amount of acid present. A brown, brownish-yellow, or brownish-red color always indicates that excessive heat has been applied or that free hydrochloric acid is absent.

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[1] Günzburg, Centralbl. f. klin. Med., 1887, vol. viii, No 40.

Organic acids do not produce the reaction, nor is it interfered with by their presence, or that of albumins, peptones, or acid salts.

A phloroglucin-vanillin test paper, prepared by soaking strips of filter paper, free from ash, in the solution and drying them, may also be employed. If a strip of this is moistened with a drop of gastric juice and gently heated in a porcelain dish, the rose color will develop in the presence of free hydrochloric acid, and does not disappear upon the addition of ether.

The Resorcin Test.[1]—The solution consists of 5 grams of resublimed resorcin and 3 grams of cane sugar dissolved in 100 grams of 94 per cent. alcohol. It is equally as delicate as the phloroglucin-vanillin solution and has the advantage of greater stability: 5 or 6 drops of gastric juice are treated with 3 to 5 drops of the reagent and slowly evaporated to dryness over a small flame, when a beautiful rose- or vermilion-red mirror will be obtained, which gradually fades on cooling. If the reagent is employed in the form of a test paper, a violet color at first develops, which upon the application of heat turns brick red and does not disappear on treatment with ether.

The presence of acid salts, organic acids, albumins, or albumoses does not interfere with the reaction.

The Tropæolin Test.[2]—Tropæolin 00, when employed according to the method suggested by Boas, is a very reliable reagent, indicating the presence of 0.2 to 0.3 pro mille of free hydrochloric acid: 3 or 4 drops of a saturated alcoholic solution of tropæolin 00, which has a brownish-yellow color, are placed in a small porcelain dish or cover, and allowed to spread over the surface. A like amount of gastric juice is added and likewise allowed to flow over the surface of the dish; upon the application of gentle heat a beautiful lilac appears, which is said to be characteristic of free hydrochloric acid.

A tropæolin test paper may also be prepared by soaking filter paper, free from ash, in the alcoholic solution, and then drying and cutting it into strips. A few drops of gastric juice containing free hydrochloric acid produce a more or less pronounced brown color upon this paper, which turns lilac or blue upon the application of gentle heat. Organic acids, when present in large amounts, likewise produce a brown color, but this disappears on heating, and a lilac or blue color does not result.

For ordinary purposes this test is sufficient, and recourse need only be had to the more delicate reagents when a negative or a doubtful result is obtained.

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[1] Boas, Centralbl. f. klin. Med., 1888, vol. ix, No. 45.

[2] Ewald, Klinik. d. Verdauungskrank., Berlin, 1888, vol. ii; and Boas, Deutsch. med. Woch., 1877, vol. xiii, p. 852.

The Combined Hydrochloric Acid.—It has been pointed out elsewhere that hydrochloric acid will only appear in the free state after all basic affinities have been saturated. For this reason combined hydrochloric acid must of necessity be present after the administration of a test meal if free acid can be demonstrated. If the contents are withdrawn too early free acid will be absent, while hydrochloric acid in combined form may be present in normal amount, considering the stage of digestion. From the mere absence of free hydrochloric acid it is hence not justifiable to infer that no hydrochloric acid has been secreted. Under pathological conditions it may happen that while the stomach has lost the power to furnish a sufficient amount of hydrochloric acid to satisfy the albuminous affinities of a large meal and to subsequently appear in the free state, enough can be furnished to meet the demands of a small meal. In any case then, where free hydrochloric acid is not found, it is important to ascertain whether no hydrochloric acid at all has been secreted. To this end the method of Martius and Lüttke may be employed (see below).

Quantitative Estimation of the Hydrochloric Acid of the Gastric Juice. Töpfer's Method.[1]—The free and combined hydrochloric acid is most conveniently estimated according to Töpfer's method, which is both simple and sufficiently accurate for clinical purposes.

In this method the total acidity (*a*) of a given amount of gastric juice—*i. e.*, the acidity referable to the presence of free hydrochloric acid, combined hydrochloric acid, acid salts, and any organic acids that may be present—is first determined (lactic acid and the fatty acids, if present, need not be removed), using phenolphthalein as an indicator. This is followed by a determination of the acidity referable to free acids and acid salts in another sample of gastric juice (*b*), using alizarin (alizarin monosulphonate of sodium) as an indicator. As this does not react with loosely combined hydrochloric acid, the difference between *a* and *b* will indicate the amount of the latter. The free hydrochloric acid (*c*) finally is estimated with dimethyl-amido-azo-benzol as an indicator, the difference between *a* and $b+c$ giving the acidity referable to organic acids and acid salts.

The solutions required are the following:

1. A decinormal solution of sodium hydrate.
2. A 1 per cent. alcoholic solution of phenolphthalein.
3. A saturated aqueous solution of alizarin.
4. A 0.5 per cent. alcoholic solution of dimethyl-amido-azo-benzol.

Three separate portions of 5 or 10 c.c. of filtered gastric juice are measured into three small beakers or porcelain dishes. To the first portion 1 or 2 drops of phenolphthalein are added, when it is titrated with the one-tenth normal solution of sodium hydrate until a permanent pink color is obtained.

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[1] Loc. cit.

To the second portion 3 or 4 drops of the alizarin solution are added, when it also is titrated with the one-tenth normal solution of sodium hydrate until a pure violet color is obtained (Plate XII, Fig. 3).

In the third portion the free hydrochloric acid is titrated, after the addition of 3 or 4 drops of the dimethyl-amido-azo-benzol, until the last trace of red—in the presence of free hydrochloric acid—has disappeared, and the color has become distinctly greenish yellow (Plate XII, Fig. 2). The results are then calculated as in the following example:

10 c.c. of gastric juice, using phenolphthalein as an indicator, required 6 c.c. of the one-tenth normal solution in order to bring about the end reaction, while a like amount titrated in the same manner with alizarin required 3 c.c. The difference between 6 and 3 indicates the number of cubic centimeters necessary to neutralize the amount of hydrochloric acid in combination with albuminous material. In the estimation of the free hydrochloric acid 2.3 c.c. of the one-tenth normal solution were required.

The results can then be tabulated as follows:

Total acidity (per 100 c.c. stomach contents)	60
Alizarin acidity	30
Combined hydrochloric acid	30
Free hydrochloric acid	23
Total physiologically active hydrochloric acid	53
Salts	7
Total	60

If not enough gastric juice is available for three separate titrations one can estimate the free hydrochloric acid in one portion of 5 c.c. with dimethyl as an indicator, and proceed at once to the total acidity in the same example. To this end phenolphthalein is added after the primary titration and the titration continued for the total acidity as usual. The first value will give the free hydrochloric acid and this plus the second value the total acidity.

Deficit of Hydrochloric Acid.—When hydrochloric acid is absent it is customary to indicate the deficit in terms of $\frac{n}{10}$ hydrochloric acid in a manner perfectly analogous to the method just now described, viz., 10 c.c. of gastric juice are treated with a few drops of dimethyl and then titrated with $\frac{n}{10}$ hydrochloric acid until the red hydrochloric acid reaction appears. If 1 c.c. was necessary to this end the hydrochloric acid deficit would be 10.

Estimation of Free Hydrochloric Acid (according to Sahli).—25 to 30 drops of Günzburg's reagent are added to 10 c.c. of gastric juice.

The mixture is titrated with a decinormal sodium hydrate solution as usual until a drop of the mixture, warmed on the stirring rod after each addition of the alkali, shows a red color. The rod must be washed and cooled after every test.

The Method of Martius and Lüttke (modified).[1]—This method is equally exact, but requires a greater expenditure of time. It is based upon the fact that upon incineration of the gastric juice the free hydrochloric acid and that loosely combined with albuminous material escape, while the chlorine in combination with inorganic bases remains in the mineral ash unless a very intense heat is applied for some time. By subtracting the amount of chlorine present in the latter form from the total amount, the quantity in combination with albuminous material and that occurring as free acid will be found. The total acidity of the gastric juice is then determined, and that referable to the presence of the free and combined hydrochloric acid subtracted, the difference giving the amount of organic acids and acid salts. By determining the acidity due to the presence of free hydrochloric acid according to Töpfer's method, and deducting the amount found from that referable to the presence of free and combined hydrochloric acid, the amount of the latter is obtained.

Reagents required:

1. A solution of silver nitrate in nitric acid of such strength that 1 c.c. shall represent 0.00365 gram of hydrochloric acid.
2. Liquor ferri sulphurati oxydati.
3. A decinormal solution of ammonium sulphocyanide.
4. A one-tenth normal solution of sodium hydrate.
5. A 1 per cent. alcoholic solution of phenolphthalein.
6. A 0.5 per cent. alcoholic solution of dimethyl-amido-azo-benzol.

Preparation of the solutions:

1. The silver nitrate solution. As a solution is required of such strength that 1 c.c. shall be equivalent to 0.00365 gram of hydrochloric acid, the amount of silver nitrate that must be dissolved in 1000 c.c. of water is ascertained in the following manner: Since 169.66 (molecular weight) parts by weight of silver nitrate combine with 36.5 parts of hydrochloric acid (molecular weight), the amount of silver nitrate required for each cubic centimeter is found from the equation

$$169.66 : 36.5 :: x : 0.00365;\ 36.5\ x = 0.6192590;\ x = 0.0169.$$

In 1 c.c. of the silver solution 0.0169 gram of silver nitrate must thus be present, or 16.9 grams in the liter. This quantity, or roughly 17 grams, is weighed off and dissolved in 900 c.c. of a 25 per cent. solution of nitric acid. To this solution 50 c.c. of the liquor ferri sulphurati oxydati are added. The solution is then brought to the proper strength by titration of a known number of cubic centimeters of a one-tenth normal solution of hydrochloric acid and correcting as usual (see below).

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[1] Die Magensäure des Menschen, Stuttgart, 1982.

2. The ammonium sulphocyanide solution. A normal solution of ammonium sulphocyanide contains 75.98 grams (molecular weight) per liter, and a decinormal solution 7.598 grams. This quantity, or roughly 8 grams, is dissolved in about 900 c.c. of water and the solution brought to the proper strength by titrating a known number of cubic centimeters of the silver nitrate solution, when ach cubic centimeter should correspond to 1 c.c. of the silver solution —*i. e.*, to 0.00365 gram of hydrochloric acid. It is corrected as described elsewhere (see below).

METHOD.—1. To determine the total amount of chlorine present: 10 c.c. of filtered gastric juice—Martius and Lüttke make use of the unfiltered gastric contents—are measured into a small flask bearing a 100 c.c. mark, and treated with an excess of the one-tenth normal solution of silver nitrate. Experience has shown that 20 c.c. are sufficient. The mixture is agitated and allowed to stand for ten minutes. Distilled water is then added to the 100 c.c. mark; the mixture is agitated once more and filtered through a dry filter into a dry beaker; 50 c.c. of the filtrate are titrated with the one-tenth normal solution of ammonium sulphocyanide until the blood-red color which appears upon the addition of every drop—due to the formation of ferric sulphocyanide—no longer disappears on stirring. By multiplying the number of cubic centimeters of the ammonium sulphocyanide solution used by 2 (the number of cubic centimeters that would have been necessary for the precipitation of the excess of silver in 100 c.c.) and deducting the result from the number of cubic centimeters of the one-tenth normal solution of silver nitrate employed, viz., 20, the number of cubic centimeters of the latter solution is found which was necessary to precipitate the chlorine in 10 c.c. of the gastric juice. As 1 c.c. of the solution represents 0.00365 gram of hydrochloric acid, it is only necessary to multiply this figure by the number of cubic centimeters used in precipitation of the chlorine. The resulting value, *T*, expresses the total amount of chlorine present.

As a general rule, it is not necessary to decolorize the gastric juice. If desired, however, 5 to 15 drops of a 5 per cent. solution of potassium permanganate may be added to the 10 c.c. employed, after the mixture has stood for ten minutes.

2. Determination of the amount of chlorine in combination with inorganic bases, *F:* 10 c.c. of the filtered gastric juice are carefully evaporated to dryness in a platinum crucible, on a water bath or upon a plate of asbestos, in order to avoid sputtering (as the heat applied in the process of incineration is not very intense, a porcelain crucible may also be employed). The residue is then care-

fully incinerated over an open flame, the process being carried only to the point where the organic ash no longer burns with a luminous flame. Intense heat should be avoided, as the chlorides are volatilized upon the application of red heat. On cooling, the ash is moistened with a few drops of distilled water and mixed with a stirring rod, when the residue is extracted in separate portions with 100 c.c. of hot distilled water and filtered. This amount is usually sufficient to dissolve all the chlorides present. If any doubt should exist, however, it is only necessary to add a drop of the silver solution to a few drops of the last portion of the filtrate: the formation of a cloud, referable to silver chloride, will necessitate still further washing. The whole filtrate is then treated with 10 c.c. of the one-tenth normal solution of silver nitrate, and the amount consumed in the precipitation of the chlorides determined by titration with the one-tenth normal solution of ammonium sulphocyanide, as described above. The hydrochloric acid present in combination with inorganic bases is thus determined. The difference between the amount of hydrochloric acid in combination with inorganic bases and the total amount of chlorine in terms of hydrochloric acid will then indicate the amounts of the free and of the combined hydrochloric acid, which are termed L and C, respectively; hence $T—F = L+C$.

3. The total acidity in terms of hydrochloric acid is further determined according to the method given elsewhere (see p. 220) and indicated by the letter A. The difference between the total acidity and the amount of free and combined hydrochloric acid will represent the amount of organic acids and acid salts, O; hence $O=A—(L+C)$.

The free hydrochloric acid finally is determined according to the method of Töpfer. The difference between the value thus found and that expressing the amount of free and combined hydrochloric acid will indicate the amount of the latter; hence $(L+C)—L=C$.

Leo's Method.[1]—This method is based upon the observation that calcium carbonate combines with free and combined hydrochloric acid at ordinary temperatures to form neutral calcium chloride, while the acid phosphates are not affected. It is thus clear that by determining the total acidity of the gastric juice, and deducting from this the acidity referable to acid salts, the amount of the physiologically active hydrochloric acid—*i. e.*, of the free and combined hydrochloric acid—is obtained.

As it has been shown that in the presence of calcium chloride (formed, as indicated above, upon the addition of calcium carbonate), owing to the formation of calcium monophosphate—$CaHPO_4$, twice the quantity of sodium hydrate is taken up, it is necessary to make the first titration also after the addition of an excess of calcium chloride.

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[1] Centralbl. f. d. med. Wiss., 1889, vol. xxvii, p. 481.

Reagents required:

1. A one-tenth normal solution of sodium hydrate.
2. A 1 per cent. alcoholic solution of phenolphthalein.
3. A concentrated solution of calcium chloride.
4. Chemically pure calcium carbonate. The purity of the salt may be tested by stirring a small piece with water; the solution should not color red litmus paper blue. A solution of the salt in dilute hydrochloric acid should not yield a precipitate when treated with sulphuric acid.

METHOD.—Organic acids that may be present are first removed by shaking with ether, 50 to 100 c.c. being required for each 10 c.c. of gastric juice. The total acidity of the gastric juice is then determined in 10 c.c. of the filtered liquid after the addition of 5 c.c. of the concentrated solution of calcium chloride, the result being termed A.

The acidity referable to the presence of acid phosphates is determined as follows: 15 c.c. of filtered gastric juice are treated with a pinch of dry and chemically pure calcium carbonate; the mixture is thoroughly stirred, and passed at once through a dry filter; 10 c.c. of the filtrate, from which the carbon dioxide is expelled by means of a current of air, are then treated with 5 c.c. of the calcium chloride solution and titrated as above, the resulting value being termed P. $A—P$ is hence equivalent to $L+C$. The value of C can then be ascertained by determining the acidity referable to free hydrochloric acid according to Töpfer's method, and deducting the value found from $L+C$.

This method is sufficiently accurate for practical purposes, and has the advantage of not requiring the expenditure of much time.

The Ferments of the Gastric Juice and their Zymogens.

Normal gastric juice contains three ferments, viz., pepsin, chymosin, and lipase.

Pepsin and Pepsinogen.—According to our present knowledge, the zymogen of pepsin, viz., pepsinogen or propepsin, and not pepsin itself, is secreted by the chief cells of the fundus glands. It is transformed into the ferment proper by the hydrochloric acid of the gastric juice.

This is not the place to enter into a detailed consideration of the various properties of pepsin, and it will suffice to say that the activity of the ferment is destroyed by even very dilute solutions of the alkaline carbonates. The same result is reached by exposing a watery solution of pepsin to a temperature of 70° C., while in a dry state a temperature of 100° C. will not destroy its activity; this is shown by the fact that a specimen of pepsin thus treated is, on cooling, still capable of digesting albumins in the presence of hydrochloric acid.

While pepsin is capable of digesting albumins in the presence of other acids, viz., phosphoric, sulphuric, oxalic, acetic, lactic, and salicylic acids, the solutions must be stronger than in the case of hydrochloric acid. With lactic acid, for example, a satisfactory result is reached only with a concentration of from 12 to 18 pro mille, while of hydrochloric acid 2 to 4 pro mille are sufficient. Larger or smaller amounts do not act so promptly.

Figures expressing the exact quantity of pepsin or of its zymogen are lacking, and inferences can hence only be drawn as to the physiological activity of the ferment from the rapidity with which given amounts of albuminous material are digested. This, however, depends to a large extent upon the nature and concentration of the free acid present. Under normal conditions 25 c.c. of gastric juice will dissolve 0.05 to 0.06 gram of serum albumin in one hour, the same amount of coagulated egg albumen in three hours, and a like amount of fibrin in one hour and a half.

As abnormalities in the circulation and innervation of the stomach apparently do not influence the production of pepsin, or rather of its zymogen, a diminution in the degree of peptic activity, or its total absence, may be referred directly to disease of the stomach itself, viz., its glandular apparatus. The determination of the presence or absence and relative amount of pepsin in the gastric juice hence furnishes more useful information than the recognition of the presence or absence of free hydrochloric acid.

As pepsin is formed from pepsinogen through the agency of a free acid, its presence, in the absence of organic acids in notable quantities, indicates at once the presence of hydrochloric acid. It may be said, *vice versa*, that if free hydrochloric acid is present in the gastric juice pepsin also will be found. Should the zymogen alone be present, digestion will take place only upon the addition of an acid, while an absence of digestion upon the addition of hydrochloric acid indicates the absence of both pepsin and its zymogen. At times, though rarely, a "gastric juice" is met with which is capable of digesting albumin in the absence of hydrochloric acid, owing to the presence of regurgitated pancreatic juice.

In the differential diagnosis of a chronic gastritis and a neurosis, or a dyspeptic condition referable to hyperemia of the gastric mucous membrane, the demonstration of zymogen in the absence of hydrochloric acid may, at times, be very important, bearing in mind that circulatory and nervous disturbances apparently do not influence the production of pepsinogen. An entire absence of the latter would, of course, warrant the diagnosis of anadeny of the stomach.

Tests for Pepsin and Pepsinogen. TEST FOR THE ENZYME.—If the presence of free hydrochloric acid has previously been ascertained, 25 c.c. of filtered gastric juice are set aside and kept at a temperature of from 37° to 40° C., a bit of coagulated egg albumen, fibrin, or serum albumin being added. In order to permit of a comparison of results, the same amounts should always be taken; 0.05 to 0.06 gram of egg albumen, as has been shown, ought, under physiological conditions, to be digested in three hours.

TEST FOR THE ZYMOGEN.—Should hydrochloric acid be absent the test is made in the same manner, after the addition of from 3 to 5 drops of the officinal solution of hydrochloric acid to 25 c.c. of the filtrate. Under such conditions usually pepsinogen alone is found.

Quantitative Estimation of Pepsin.—Accurate methods for the quantitative estimation of pepsin are unknown, and relative values only can be obtained.

Hammerschlag's Method.[1]—Two Esbach tubes (albuminimeters) are employed. Tube *A* is filled to the mark *U* with a mixture of 10 c.c. of a 1 per cent. solution of egg albumen[2] in 0.4 per cent. of hydrochloric acid and 5 c.c. of filtered gastric juice. The second tube, *B*, receives a mixture of the same solution and 5 c.c. of water. After the tubes have been kept in the thermostat for one hour at a temperature of 37° C. Esbach's reagent (see Urine) is added to each tube to the mark *R*. After standing for twenty-four hours the amount of precipitated albumen is read off in the two tubes. The difference indicates the amount of albumen which was digested; this raised to the square gives the corresponding amount of pepsin (which of course is merely relative). The method suffices for practical purposes.

Mett's Method.—Satisfactory comparative results can also be obtained with the method suggested by Mett. Capillary glass tubes are prepared measuring from 1 to 2 mm. in diameter. They are filled with white of egg, closed at the ends with breadcrumbs and coagulated in boiling water. After five minutes they are dried and the ends closed with melted paraffin. In this form they can be kept, but before use they should be examined to see that the column of albumen has not shrunk from the sides. Any bubbles that may be present disappear after two days. When needed they are cut into pieces from 1 to 2 cm. long. The length of the column digested in a given length of time serves as a measure of the digestive power of the specimen examined. In practice this column should be measured in millimeters with the aid of a magnifying glass, or a low power of the microscope, using a stage micrometer. The calculation of the corresponding amount of ferment is based upon the law of Schütz and Borrissow, viz., that the corresponding amounts of ferment in two solutions bear the same ratio toward each other as the square of the number of millimeters of the column of egg albumen which has been dissolved in the same length of time. Nirenstein and Schiff[1] have ascertained that the length of the digested cylinder of albumen is proportionate to the length of time that digestion goes on, providing that the length of the cylinder does not exceed 7 mm. If it does exceed this, digestion proceeds more slowly. It is hence advisable in all cases to dilute the gastric juice. In this manner another difficulty also is obviated, viz., the antipeptic activity which is caused by certain substances which are normally present in solution (products of digestion, sodium chloride). Nirenstein and Schiff ascertained that a sixteenfold dilution with $\frac{n}{20}$ HCl (0.18 per cent.) is sufficient and that this prevents the digestion of more than 3.6 mm. in twenty-four hours, which is a further condition to ensure reliable results.

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[1] Wien. med. Presse, 1894, vol. xxxv, p. 1654.

[2] The white of one egg diluted about 13 times will make a 1 per cent. solution.

METHOD.—The gastric juice is obtained after giving Ewald's test breakfast. 1 c.c. of the filtered contents is diluted with 16 c.c. of $\frac{n}{20}$ HCl; into this solution 4 Mett's tubes are placed and the mixture is kept in the incubator for twenty-four hours. The columns of digested albumen are measured and the average ascertained; this in terms of millimeters raised to the square and multiplied by 16 (the degree of dilution) indicates the relative amount of pepsin. If the digested column measures more than 3.6 mm. the gastric juice must be diluted thirty-two times.

The unit of measure is the amount of pepsin by which 1 mm. of albumen is digested in twenty-four hours, with an acidity of 0.18 per cent. HCl. Nirenstein and Schiff in their series found variations from 0 to 256 pepsin units.

Quantitative Estimation of Pepsinogen.—In order to estimate the amount of pepsinogen both Hammerschlag's and Mett's method can be applied after rendering the gastric contents acid with hydrochloric acid to the extent of from 1 to 2 pro mille.

The Milk-curdling Ferment and its Zymogen, viz., Chymosin (Rennin) and Chymosinogen.—The specific action of chymosin is exerted upon milk, or lime-containing solutions of casein, which are coagulated in neutral or feebly alkaline solutions.

In this connection it is important to note that the addition of a few cubic centimeters of a solution of calcium chloride, or any other soluble lime salt, results in a transformation of the zymogen into the physiologically active ferment, and that hydrochloric acid, while it normally causes such transformation, is not absolutely necessary in the presence of calcium chloride.

Under physiological conditions chymosin and its zymogen are always present in the gastric juice. In disease the inferences that may be drawn from a quantitative estimation of the ferment and its zymogen have been formulated by Boas,[1] to whom we are indebted for much valuable information in this connection:

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[1] Arch. f. Verdauungsk., 1903, vol. viii.

1. Notwithstanding the absence of free hydrochloric acid, chymosin may be present, although in minimal traces—*i. e.*, demonstrable with a dilution of from 1 to 10 to 1 to 20 (see method below).
2. In the absence of free hydrochloric acid the zymogen may still be present in normal amounts—*i. e.*, demonstrable with a dilution of from 1 to 100 to 1 to 150. The presence of the zymogen, especially when repeatedly observed, probably always permits of the conclusion that we are not dealing with an organic disease of the stomach, but with a neurosis or a hyperemic condition of the mucous membrane referable to disease of other organs.
3. The zymogen may occur in moderately diminished amount, 50 per cent. only being present. This is usually owing to the existence of a gastritis which has not reached its highest degree of severity. The nearer the amount of zymogen approaches the normal, the greater will be the probability of an ultimate recovery under suitable treatment.
4. The amount of the zymogen is greatly diminished (dilutions of 1 to 10 to 1 to 25 yielding a negative result) or may be absent altogether. In cases of this kind a severe and usually incurable gastritis exists, either primary or occurring secondarily to carcinoma, amyloid degeneration, etc.
5. In conditions 1, 2 and 3, the reëstablishment of the secretion of hydrochloric acid may be attempted with some prospect of success by means of stimulating remedies.

These conclusions are based upon the employment of Ewald's test breakfast, and cannot be applied to observations made after other test meals, without previous studies in this direction.

Testing for the presence of chymosin and its zymogen is of decided value in cases in which alkaline material is vomited, and where we may be called upon to decide whether this contains constituents of the gastric juice or not.

Tests for Chymosin and Chymosinogen. TEST FOR THE ENZYME.—5 to 10 c.c. of milk are treated with 3 to 5 drops of the filtered gastric juice and kept at a temperature of 37° to 40° C. for ten to fifteen minutes. If coagulation occurs during this time, it may be concluded that the enzyme is present.

TEST FOR THE ZYMOGEN.—The milk is treated with 10 c.c. of the filtered and feebly alkalinized gastric juice and with 2 or 3 c.c. of a .1 per cent. solution of calcium chloride. The mixture is kept at a temperature of from 37° to 40° C., when in the presence of the zymogen the formation of a thick cake of casein will occur within ten to fifteen minutes.

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[1] Centralbl. f. d. med. Wiss., 1887, vol. xxv, p. 417; and Zeit f. klin. Med., 1888, vol. xiv, p. 240. See also J. Friedenwald, Med. News, 1895.

Quantitative Estimation. OF THE ENZYME.—The method is based upon the fact that on gradually diluting a specimen of gastric juice a point is finally reached at which a chymosin reaction can no longer be obtained, the value being, of course, a relative one. Under physiological conditions a positive reaction can still be observed with a degree of dilution varying between 1 to 30 and 1 to 40.

The gastric juice is neutralized with a very dilute solution of sodium hydrate. Tubes are then prepared containing from 5 to 10 c.c. of the gastric juice, diluted in the proportion of 1 to 10, 1 to 20, 1 to 30, etc., to which an equal amount of neutral or amphoteric milk is added. The tubes, properly labelled, are kept at a temperature of from 37° to 40° C., and the degree of dilution noted at which coagulation still occurs.

OF THE ZYMOGEN.—The gastric juice is rendered feebly alkaline and tubes are prepared containing equal amounts of milk and gastric juice, the latter variously diluted, as above directed; the examination is then carried on in the same manner. Normally a positive reaction is obtained with a dilution varying between 1 to 150 and 1 to 100.

Lipase.—The presence of lipase as a normal constituent of the gastric juice has now been definitely established. Its demonstration and quantitative estimation are described in the section on the Urine. It is essential that the examination be made after a thorough washing of the stomach and the administration of a test meal which is free from fat.

Analysis of the Products of Albuminous Digestion.

In order to separate the various products of digestion from each other the following procedure may be employed:

The filtered gastric contents are carefully neutralized with a dilute solution of sodium hydrate, using litmus paper to determine the reaction; a small drop of the mixture is placed upon the paper from time to time during the addition of the sodium hydrate until no change in color is produced either on the red or the blue paper. If syntonin is present, it will be precipitated, and can be collected on a small filter. Upon the addition of an excess of dilute acid or an alkali this precipitate will again be dissolved. The filtrate is feebly acidified with dilute acetic acid, treated with an equal volume of a saturated solution of common salt, and brought to the boiling point. Any native albumin that may be present in solution is thus coagulated and can be filtered off on cooling. In the filtrate the albumoses and peptones remain.

By one-half saturation of the filtrate with ammonium sulphate, viz., by adding an equal amount of a saturated solution of ammonium sulphate, the primary albumoses can be precipitated. If then the neutral filtrate is treated with one-half its volume of a saturated solution of ammonium sulphate, which will thus give a two-third total saturation, a portion of the deutero-albumoses (fraction *A*) separates out on standing. This is filtered off and the solution saturated with ammonium sulphate in substance; the deutero-fraction *B* is thus thrown down, and on acidifying the filtrate with one-tenth of its volume of a solution of sulphuric acid that has been saturated with ammonium sulphate, and of which 10 c.c. correspond in strength to 17 c.c. of a $\frac{n}{10}$ solution of sodium hydrate, the last traces of deutero-albumoses (fraction *C*) will separate out on standing.

The filtrate contains the "peptones." To demonstrate these a 2 per cent. solution of cupric sulphate is added drop by drop, when in the presence of peptones a rose- to a purplish-red color will develop.[1]

Tests for the Products of Carbohydrate Digestion.

Starch may be recognized by the fact that it strikes a blue color with a solution of iodopotassic iodide, while the same solution gives a violet or mahogany brown with erythrodextrin. To this end it is only necessary to add a drop or two of Lugol's solution to a few cubic centimeters of the filtered gastric juice. The presence of achroödextrin may be inferred if no change in color occurs upon the addition of the reagent.

Maltose and dextrose, which both react with Fehling's solution and undergo fermentation, differ from each other in the fact that the former does not reduce *Barfoed's reagent* on boiling. This is prepared by adding 1 per cent. of acetic acid to a 0.5 to 4 per cent. solution of cupric acetate. The rotatory power of maltose is about three times as strong as that of dextrose; $(\alpha)\ D = 150.4$, as compared with 52.5.

Lactic Acid.

Mode of Formation and Clinical Significance.—The normal occurrence of lactic acid in the stomach during digestion was until recently regarded as an established fact and generally ascribed to the action of lactic acid producing organisms which had been swallowed and which could exercise their activity so long as hydrochloric acid did not appear in the free state.

Martius and Lüttke, however, employing the method already described, found "that the accurately determined curve of acidity referable to hydrochloric acid coincided in all respects, even at the beginning of the process of digestion, with the curve referable to the total acidity," so that lactic acid as a physiological constituent could not have been present. The researches of Boas,[1] moreover, prove beyond a doubt that in physiological conditions no appreciable amounts of lactic acid are formed during the process of digestion, and that the lactic acid found after an ordinary meal has been introduced into the stomach as such. It is known that lactic acid is present in various kinds of bread and it is, hence, not permissible to make use of any test meal containing lactic acid when the question as to its formation in the stomach is to be considered. For these reasons Boas suggests the use of simple oatmeal soup to which salt only has been added. For practical purposes this is probably not always necessary, as the small amount of lactic acid found after Ewald's test breakfast may usually be disregarded; an increased amount can be referred directly to pathological conditions.

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[1] For a more detailed account of the chemistry of digestion and the analysis of the resulting products, see C. E. Simon, Physiological Chemistry, Lea Bros. & Co.

The fact that the lactic acid disappears or is at least no longer demonstrable at the height of digestion may be due to its resorption on the one hand, or to an interference of the hydrochloric acid with the delicacy of the reagent usually employed—*i. e.*, Uffelmann's reagent—on the other.

Under pathological conditions notable amounts (1 to 4 pro mille) of lactic acid are met with when stagnation of the gastric contents occurs as a result of motor insufficiency, in the absence of or with a diminished secretion of hydrochloric acid. It is hence a common symptom of carcinoma of the stomach.[2] It was indeed at one time thought that carcinoma was the only disease in which a notable lactic acid production took place, but experience has shown that the same may occur in benign cases of pyloric stenosis and gastric insufficiency. Such findings, however, are uncommon, and a high lactic acid value may still be regarded as strongly suggestive of malignant disease and especially when repeatedly observed. Early in the disease it appears that periods of chlorhydria and lactic acid production may alternate and it is desirable that this phase of the problem more particularly receive attention.

In cases in which carcinoma has developed upon the basis of an old ulcer, lactic acid may be absent and hydrochloric acid present in increased amount.

In every case in which lactic acid is found the stomach should be thoroughly washed out in the evening and no food allowed until the fol-

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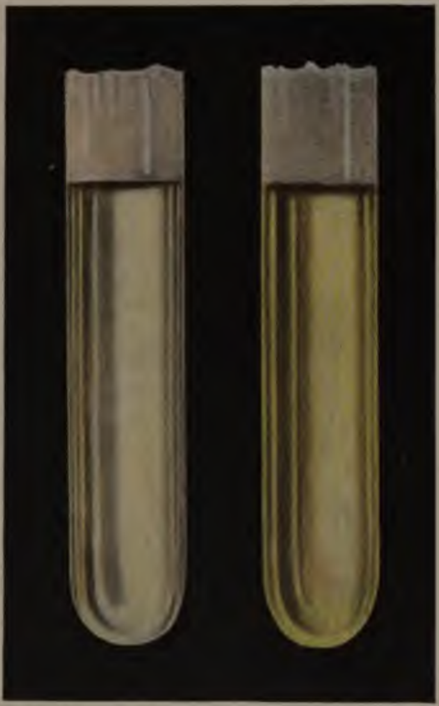
[1] "Ueber d. Vorkommen v. Milchsäure im gesunden u. kranken Magen," Zeit. f. klin. Med., 1894, vol. xxv, p. 285.

[2] J. H. de Jong, "Der Nachweis d. Milchsäure u. ihre klinische Bedeutung," Arch. f. Verdauungskrank., vol. ii, p. 53. J. Friedenwald, "The Significance of the Presence of Lactic Acid in the Stomach," N. Y. Med. Jour., 1895. Rosenheim u. Richter, "Ueber Milchsäurebildung im Magen," Zeit. f. klin. Med., vol. xxviii, p. 505.

PLATE XIII.



Kelling's Test for Lactic Acid.

lowing morning. Boas' test meal is then given and the examination repeated. If the presence of lactic acid can thus be established on repeated examination, even if a normal condition or hyperchlorhydria can be demonstrated in the interval, an exploratory incision is justifiable.

It should, finally, be mentioned that only that form of lactic acid which results from fermentative processes is of interest in this connection, and not the sarcolactic acid contained in meat. For this reason the demonstration of lactic acid after a meal of meat is of no diagnostic significance, so far as the question of carcinoma goes.

Kelling's Method[1] (Author's Modification).—This test is best performed in the following manner: A test tubeful of water receives a drop or two of a moderately strong solution of the sesquichloride of iron, so that the liquid is barely colored. One half is then poured into a second tube and serves as control. A small amount of the gastric filtrate is added to the other specimen, when in the presence of lactic acid a distinct yellow develops at once, which appears the more marked when compared with the nearly colorless control. This test is very delicate and to be preferred to the older method of Uffelmann. (Plate XIII).

Uffelmann's Test.[2]—Heretofore Uffelmann's reagent was quite commonly employed in testing for lactic acid, but everyone who has had occasion to make frequent use of this reagent in clinical work must have been struck with the uncertainty of the results so often obtained. In a large majority of the cases, particularly if Ewald's test breakfast is employed, a characteristic reaction—*i. e.*, the occurrence of a lemon or canary-yellow color—is not seen, notwithstanding the presence of lactic acid, but a pale yellow, brownish, grayish white, or even gray color is obtained instead, often leaving in doubt whether lactic acid is present or not. Aside from doubtful results, the value of the test is greatly diminished by the fact that glucose, acid phosphates, butyric acid, and alcohol give the same reaction, and that in the presence of such amounts of hydrochloric acid as are found at the height of normal digestion lactic acid is not indicated by the reagent. All these difficulties have long been appreciated, and in order to obviate at least some of them it was proposed to apply the test to an aqueous solution of the ethereal extract of the gastric contents:

To this end 5 or 10 c.c. of the filtered gastric juice are extracted by shaking with from 50 to 100 c.c. of neutral sulphuric ether[3] in a stoppered separating funnel for about twenty or thirty minutes; the ethereal extract is then evaporated on a water bath or the ether distilled off (*no flame*). The residue is taken up with from 5 to 10 c.c. of distilled water and tested as follows: 3 drops of a saturated aqueous solution of ferric chloride are mixed with 3 drops of a concentrated solution of pure carbolic acid and diluted with water until an amethyst-blue color is obtained; to this solution a portion of the ethereal extract is added, when in the presence of only 0.1 per cent. of lactic acid a lemon or canary-yellow color is obtained.

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[1] "Rhodan im Mageninhalt; Zugleich ein Beitrag z. Uffelmann'schen Milchsäurereagens," Zeit. f. physiol. Chem., vol. xviii.

[2] Deutsch. Arch. f. klin Med., 1880, vol. xxvi; and Zeit. f. klin. Med., vol. viii, p. 392.

[3] If lactic acid is not present in the free state, but in combination with albumin (*i. e.*, if the Congo-red test is negative), it is necessary to set it free by adding dilute hydrochloric acid until the Congo test is just positive, as the ether will otherwise not extract it.

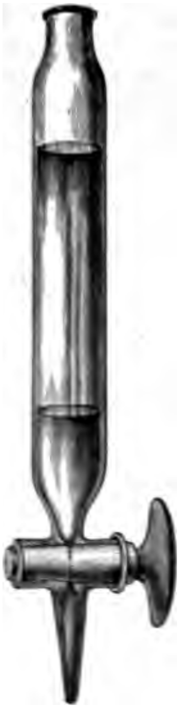

FIG. 67. Strauss' apparatus for the approximative estimation of lactic acid.

Strauss' Method.[1]—Instead of evaporating the ether as in the above method, the ethereal extract may be directly examined by shaking with a freshly prepared solution of ferric chloride, as suggested by Fleischer. Making use of this principle, Strauss has constructed an apparatus (Fig. 67) which will be found very convenient, and which permits of roughly determining the amount of lactic acid present. The instrument is essentially a separating funnel of 30 c.c. capacity, bearing two marks, of which the one corresponds to 5 c.c., the other to 25 c.c. The apparatus is filled with gastric juice to the mark 5, when ether (free from alcohol) is added to the 25 cc. line. After shaking thoroughly, the *separated* liquids are allowed to escape by opening the stopcock until the 5 c.c. mark is reached. Distilled water is then added to the 25 mark, and the mixture treated with 2 drops of the officinal tincture of ferric chloride, diluted in the proportion of 1 to 10. Upon shaking, the water will assume an intensely green color if more than 1 pro mille of lactic acid is present, while a pale green is obtained in the presence of from 0.5 to 1 pro mille. The tincture of iron should be kept in a dark-colored dropping bottle of about 50 c.c. capacity.

It will be observed that only large amounts of lactic acid, which alone are of importance from a diagnostic point of view, are indicated by the apparatus. Small amounts, as those introduced with Ewald's test breakfast, or referable to lactic acid fermentation in the mouth, are not indicated, so that confusion as to the presence or absence of the acid can never arise.

Vournaso's Method Modification of Croner and Conheim).—The method has the advantage that extraction with ether is not necessary. It is based upon the formation of an isonitril on transforming lactic acid to iodoform and treating with an amino base. The isonitril is readily recognized by its disagreeable odor. 2 grams of potassium iodide are dissolved in a few (not more than 5) c.c. of water and 1 gram of sublimed, pulverized iodine added. The resultant solution is filtered through asbestos or glass wool and diluted to 50 c.c. with distilled water; 5 c.c. of aniline are finally added. The reagent is kept in a dark-colored bottle and must be shaken before using; it keeps for a number of months. A few c.c. of the stomach contents (diluted if necessary) are rendered strongly alkaline with 10 per cent. caustic alkali solution, boiled and treated with a few c.c. of the reagent. In the presence of lactic acid the offensive odor of isonitril appears either at once or on heating.

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[1] "Ueber eine Modifikation d. Uffelmann'schen Reaktion." Berlin. klin. Woch., 1895, No. 37.

With a dilution of 0.0025 gram in 100 c.c. the odor is still discernible.

Alcohol and acetone give the same reaction.

The chemical process which takes place is represented by the equations:

$$1.\ \underset{\text{Lactic acid.}}{2CH_3.CHOH.COOH} + 10NaOH + 12I = 4H.COONa + 6HI + 2CHI_3 + 4H_2O.$$

$$2.\ \underset{\text{Iodoform.}}{CHI_3} + 3NaOH + C_6H_5.NH_2 = 3NaI + \underset{\text{Isonitril.}}{C_6H_5NC} + 3H_2O.$$

Boas' Method.[1]—In doubtful cases the following method may be employed, as with it, and following the exhibition of Boas' test meal, all possible errors can be avoided. *The stomach must be washed perfectly clean before the test meal is introduced.*

Principle of the Method.—When a solution of lactic acid is treated with a strong oxidizing agent and heated, the lactic acid is decomposed into acetic aldehyde and formic acid, according to the equation

$$\underset{\text{Lactic acid.}}{CH_3—CH(OH)—CO.OH} = \underset{\text{Acetic aldehyde.}}{CH_3.CHO} + \underset{\text{Formic acid.}}{H.CO.OH.}$$

Practically, then, the test for lactic acid resolves itself into a test for acetic aldehyde, which can readily be recognized by testing with various reagents, such as an alkaline solution of iodopotassic iodide, Nessler's reagent, and others. Nessler's reagent is prepared as follows: 2 grams of potassium iodide are dissolved in 50 c.c. of water and treated with mercuric iodide while heating, until some of the latter remains undissolved. Upon cooling, the solution is diluted with 20 c.c. of water; 2 parts of this solution are then treated with 3 parts of a concentrated solution of potassium hydrate; any precipitate that may have formed is filtered off, and the reagent kept in a well-stoppered bottle. When aldehyde is added to such a solution a yellowish-red or red precipitate results, the exact color depending upon the amount of aldehyde present; 1 part of the aldehyde may still be recognized when diluted with 40,000 parts of water.

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[1] Deutsch. med. Woch., 1893, No. 39; and Münch. med. Woch., 1893, No. 43.

With an alkaline solution of iodopotassic iodide, aldehyde in a dilution of 1 to 20,000 will still produce a cloudiness, referable to the formation of iodoform, which is readily recognized by its characteristic odor (Lieben's test for acetone).

METHOD.—The filtered gastric juice is tested for the presence of free acids with Congo-red. If present, from 10 to 20 c.c. are evaporated to a syrup on a water bath, after the addition of an excess of barium carbonate, while the latter is unnecessary in the absence of free acids. The syrup is treated with a few drops of phosphoric acid, and the carbon dioxide removed by bringing it to the boiling point once only, when it is allowed to cool and extracted with 100 c.c. of neutral sulphuric ether (free from alcohol), by shaking for half an hour. The layer of ether is poured off after half an hour, the ether is evaporated (*no flame*), the residue taken up with 45 c.c. of water, shaken and filtered, and finally treated with 5 c.c. of sulphuric acid and a pinch of manganese dioxide in an Erlenmeyer flask. This is closed with a perforated stopper carrying a glass tube bent at an obtuse angle, the longer limb of which passes into a narrow glass cylinder containing from 5 to 10 c.c. of Nessler's reagent or a like quantity of an alkaline solution of iodopotassic iodide. If heat is now carefully applied, the aldehyde formed by the oxidation of the lactic acid with manganese dioxide and sulphuric acid passes over when the boiling point is reached, and causes the precipitation of yellowish-red aldehyde of mercury in the tube containing the Nessler reagent, or of iodoform if the alkaline solution of iodine is employed.

Quantitative Estimation of Lactic Acid According to Boas' Method.[1]—The principle already set forth also applies to the quantitative estimation of lactic acid.

Solutions required:

1. A one-tenth normal solution of iodine.
2. A one-tenth normal solution of sodium thiosulphate.
3. Hydrochloric acid (sp. gr. 1.018).
4. A potassium hydrate solution (56 to 1000).
5. Starch solution.

Preparation of these solutions:

1. A normal solution of iodine should contain 126.53 (molecular weight of iodine) grams of iodine in the liter, and a one-tenth normal solution, hence 12.6 grams. In order to dissolve the iodine 25 grams of potassium iodide are dissolved in about 200 c.c. of distilled water, when the 12.6 grams of resublimed iodine are added. This solution is then diluted with distilled water to the 1000 c.c. mark, and requires no further correction.

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[1] Loc. cit., p. 237.

2. The one-tenth normal solution of sodium thiosulphate is prepared as described in the chapter on Acetone (see Urine). When treated with 1 gram of ammonium carbonate pro liter it will retain its titre almost indefinitely.

3. Preparation of the starch solution: 5 grams of starch are dissolved in 900 c.c. of water by heating, when 10 grams of zinc chloride in 100 c.c. of water are added.

METHOD.—10 to 20 c.c. of the filtered gastric juice are first treated as indicated above, viz., evaporated to a syrup after the addition of barium carbonate if free acids are present. A few drops of phosphoric acid are added, the carbon dioxide driven off by boiling, and the residue extracted, on cooling, with 100 c.c. of ether *free from alcohol;* the ether is evaporated after separation, the residue taken up with 45 c.c. of distilled water, and treated with manganese dioxide and sulphuric acid. The flask is closed by a doubly perforated stopper; through one aperture a bent tube passes to the distilling apparatus, and a straight tube provided with a piece of rubber tubing, clamped off, through the other. The latter should dip well down into the liquid, and serves for passing a current of air through the solution when the distillation is completed. The mixture is distilled until about four-fifths of the contents have passed over, *excessive heat being carefully avoided,* as otherwise the aldehyde will be decomposed, according to the equations:

$$1.\ \underset{\text{Lactic acid.}}{CH_3.—CH(OH)—CO.OH} = \underset{\text{Aldehyde.}}{CH_3.CHO} + \underset{\text{Formic acid.}}{HCOOH.}$$

$$2.\ \underset{\text{Aldehyde.}}{CH_3.CHO} + \underset{\text{Formic acid.}}{HCOOH} + 2O = \underset{\text{Acetic acid.}}{CH_3.COOH} + CO_2 + H_2O.$$

To the distillate, which is best received in a high Erlenmeyer flask, well stoppered, 20 c.c. of the one-tenth normal solution of iodine are added mixed with 20 c.c. of the 5.6 per cent. solution of potassium hydrate. The mixture is shaken thoroughly and allowed to stand for a few minutes. In order to liberate the iodine not used in the reaction, 20 c.c. of hydrochloric acid are added, and the excess of iodine determined by titration with the one-tenth normal solution of sodium thiosulphate. The titration is carried almost to the point of decolorization, when a little starch solution is added; the mixture is then titrated until the blue color has disappeared. The number of cubic centimeters of the one-tenth normal solution employed, viz., 20, minus the number of cubic centimeters of the one-tenth normal solution of sodium thiosulphate, will then indicate the number of cubic centimeters of the former required for the formation of iodoform, viz., the amount of lactic acid present in 10 or 20 c.c. of gastric juice, as the case may be. As 1 c.c. of the one-tenth normal solution of iodine has been found to indicate the presence of 0.003388 gram of lactic acid, it is only necessary to multiply the number of cubic centimeters used by this figure, and the result by 10, in order to obtain the percentage.

The method described is reliable and sufficiently accurate for clinical purposes. At the same time it may be said that no more time is required than in the ordinary quantitative estimation of sugar by means of Fehling's method, or of hydrochloric acid according to the method of Martius and Lüttke.

BOAS' RAPID METHOD.—This method is less accurate than the preceding one, but may be advantageously employed in the absence of the various reagents necessary with the former. 10 c.c. of filtered gastric juice are treated with a few drops of dilute sulphuric acid, and the albumin present removed by heat. The filtrate is evaporated to a syrup on a water bath, water added to the original amount, and this again evaporated to a small volume, fatty acids being thereby removed. The lactic acid remaining is now extracted with ether (200 c.c. for every 10 c.c. of gastric juice); the ether is evaporated, the residue taken up with water and titrated with a one-tenth normal solution of sodium hydrate, using phenolphthalein as an indicator. As 40 parts by weight of sodium hydrate (molecular weight) combine with 90 parts by weight of lactic acid (molecular weight), and as 1 c.c. of the one-tenth normal solution of sodium hydrate contains 0.004 gram of sodium hydrate, the corresponding amount of lactic acid is found from the equation: $40 : 90 : 0.004 : x$; $40\,x = 0.360$; $x = 0.009$. The value of 1 c.c. of the one-tenth normal solution in terms of lactic acid is thus 0.009. By multiplying the number of cubic centimeters used by this figure, the amount of lactic acid present in 10 c.c. of gastric juice is ascertained. The result multiplied by 10 indicates the percentage.

The Fatty Acids.

Mode of Formation and Clinical Significance.—Unless much milk or carbohydrates have been ingested, fatty acids do not occur in the gastric contents under physiological conditions, and it would appear from the researches of Boas[1] that their formation is intimately associated with that of lactic acid. After the exhibition of his test meal he was unable to demonstrate their presence either in health or in various diseases of the stomach, such as chronic gastritis, atony or dilatation referable to benign causes, etc. In carcinoma, however, fatty acids, such as lactic acid, were quite constantly found. Flügge has shown that butyric acid can be derived from lactic acid and that this is probably its usual source.

Acetic acid fermentation presupposes the presence of alcohol, whether this is introduced into the stomach as such or whether it results from the action of yeast (Saccharomyces cerevisiæ) upon sugar. It is, hence, necessary, whenever acetic acid is met with in the gastric contents, to exclude the presence of alcohol introduced from without. Only then is it permissible to refer its presence to stagnation and advanced decomposition of carbohydrates.

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[1] Loc. cit.

If the examination is confined to an analysis of the gastric contents obtained otherwise than after the exhibition of Boas' or Ewald's test meal, the diagnosis of pyloric stenosis with dilatation is probably always justifiable in the presence of notable quantities of butyric acid and acetic acid, while the same after a previous washing out of the stomach and the exhibition of Boas' test meal would suggest carcinoma as the cause of the stenosis.

That butyric acid may occur in the gastric contents when butter or fats in general have been ingested is, of course, not surprising, and its presence then should be looked upon as a physiological occurrence. At the same time it should not be forgotten that butyric acid, just as lactic acid, may possibly have been formed in the mouth, and conclusions should, hence, only be drawn when such sources of error can be definitely excluded, and the amount found exceeds mere traces.

In conclusion it may be said that in disease butyric acid is far more frequently encountered in the gastric contents than acetic acid, but the significance of the two, if alcoholism can be excluded, is the same.

Tests for Butyric Acid.—1. Butyric acid can usually be recognized by its odor alone, which is that of rancid butter. If a more definite test is desired we may proceed as follows:

2. 10 c.c. of filtered gastric juice are extracted with 50 c.c. of ether. The ether is evaporated and the residue taken up with a few cubic centimeters of water. If a trace of calcium chloride in substance is now added the butyric acid will separate out in the form of oil droplets, the nature of which is readily recognized by the pungent odor. If, instead of adding calcium chloride, a slight excess of baryta-water is used, strongly refractive rhombic plates or granular, wart-like masses of barium butyrate are obtained upon evaporation.

3. Butyric acid may also be recognized by the peculiar odor of pineapple which develops when the dry residue of the ethereal solution is treated with a little sulphuric acid and alcohol. The reaction is due to the formation of ethyl butyrate (pineapple test).

Tests for Acetic Acid.—1. Like butyric acid, acetic acid can usually be recognized by its odor.

2. 10 c.c. of filtered gastric juice are extracted with ether. The ether is evaporated, the residue dissolved in a few drops of water, and neutralized with a dilute solution of sodium hydrate, sodium acetate being formed. If to this a drop or two of a very dilute solution of ferric chloride is added, a dark-red color results. With

silver nitrate a precipitate is obtained which is soluble in hot water.

Quantitative Estimation of the Fatty Acids. Method of Cahn-Mehring, Modified by McNaught.[1]—The total acidity is determined in 10 c.c. of filtered gastric juice. Another 10 c.c. are evaporated to a syrup, diluted with water, and similarly titrated. The difference between the two results will indicate the amount of fatty acids present.

Gases.

The stomach always contains a certain quantity of gases which have partly been swallowed and partly have passed into the stomach from the duodenum. As fermentative processes in health occur only when carbohydrates or fats have been ingested, and then only to a slight degree, nitrogen, oxygen, and carbon dioxide are the only gases found during the process of albuminous digestion. As the oxygen swallowed is, moreover, largely absorbed by the blood, and two volumes of carbon dioxide are returned for one volume of oxygen, the presence of large amounts of the former and small amounts of the latter is readily explained. In an analysis of the gases contained in the stomach of a dog which had been fed on meat, Planer found the following proportions:

Carbon dioxide	25.2	vol. per cent.
Oxygen	6.1	" "
Nitrogen	68.7	" "

With a strict vegetable diet, on the other hand, hydrogen may also be found (Planer):

	Man.		Dog.	
Carbon dioxide	20.79	33.83	32.9	vol. per cent
Oxygen	. .	0.37	0.8	" "
Nitrogen	72.50	38.22	66.3	" "
Hydrogen	6.71	27.58		

Marsh gas, CH_4, a product of the fermentation of cellulose, may also be found in pathological conditions. It is yet an open question whether marsh gas is formed in the stomach or passes into the stomach from the small intestine.

Such observations must, however, be regarded as rarities. In one case of this kind, examined by Ewald and Ruppstein,[2] in which alcohol, acetic acid, lactic acid, and butyric acid were found in the vomited material, an analysis of the gases gave the following result:

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[1] Cited by Boas, Diagnostik u. Therapie d. Magenkrankheiten, 2d ed., 1891, p. 140.

[2] Ewald, Arch. f. Anat. u. Physiol., 1874, p. 217.

Carbon dioxide	20.6	vol. per cent.
Oxygen	6.5	“ “
Nitrogen	41.4	“ “
Hydrogen	20.6	“ “
Marsh gas	10.8	“ “

Traces of olefiant gas and of hydrogen sulphide were also found. It is curious to note that in this case the patient, who, according to his own statement, had a "vinegar factory in his stomach on one day and gas works on another day," was occasionally able to light the eructated gas at the end of a cigar-holder, where it burnt with a faintly luminous flame. McNaught has reported a similar case in which the analysis furnished the following results: carbon dioxide, 56 per cent.; hydrogen, 28 per cent.; marsh gas, 6.8 per cent.; atmospheric air, 9.2 per cent.[1]

Ammonia and hydrogen sulphide are also at times met with; their presence is always due to albuminous putrefaction.

Boas[2] found that hydrogen sulphide is quite commonly present in cases of dilatation referable to benign causes, while it is almost always absent in carcinoma. He adds that it is never found when lactic acid is present. In acute gastritis it may be observed temporarily. In a number of cases of carcinoma I have never found hydrogen sulphide. In one case reported by Strauss the Bacillus coli communis was apparently concerned in its production.

To obtain a knowledge of the gases formed in the stomach during the process of digestion it is only necessary to fill an ordinary Doremus ureometer, or an Einhorn saccharimeter, with the unfiltered gastric contents, and to keep it at a temperature of from 37° to 40° C., when the evolution of gas can be followed closely and the necessary tests made. The presence of carbon dioxide is readily recognized by passing a small amount of sodium hydrate, in concentrated solution or in substance, into the tube, after the evolution has entirely ceased, when the fluid will rise. If other gases are present at the same time, they will remain after the carbon dioxide has been absorbed. Hydrogen sulphide is readily recognized by its odor and by the fact that it will color a piece of filter paper, moistened with a few drops of sodium hydrate and lead acetate, a more or less pronounced brown or black. The test is conveniently made by filling a test-tube about half-full with the gastric contents and closing it with a cork stopper to which a strip of lead paper, prepared as indicated, is fastened.

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[1] Kuhn, "Ueber Hefegährung und Bildung brennbarer Gase im menschlichen Magen," Zeit. f. klin. Med., vol. xxi; and Deutsch. med. Woch., 1892, No. 49, and 1893, No. 15.

[2] "Ueber Schwefelwasserstoffbildung im Magenkrankheiten," Centralbl. f. inn. Med., 1895, No. 3; Deutsch. med. Woch., 1892, No. 49. Zawadzki, "Schwefelwasserstoff im erweiterten Magen," Centralbl. f. inn. Med., 1894, No. 50. Dauber, "Schwefelwasserstoff im Magen," Arch. f. Verdauungskrank., vol. iv, p. 4.

Marsh gas is recognized by the fact that it burns with a scarcely luminous flame.

The eructation of gas formed in the stomach should not be confounded with the so-called *eructatio nervosa,* in which no gas is either eructated or air simply enters the esophagus and is expelled again with a loud, explosive noise. This may frequently be observed in neurasthenic and hysterical individuals, and is to a greater or less degree under the control of the will.

Acetone.

The presence of acetone in the gastric contents in pathological conditions has repeatedly been observed, especially by v. Jaksch and Lorenz,[1] and it is curious to note that the latter was at times able to demonstrate larger quantities of the substance in the gastric contents than in the urine.

In the chapter on Acetonuria the relation existing between digestive diseases and the elimination of acetone will be dealt with more fully, but it may here be mentioned that in the *primary* diseases of the gastro-intestinal tract acteone is met with quite constantly in the gastric contents, while it is observed but rarely in the secondary forms, and never is seen in the gastric neuroses. This statement, however, is denied by Sovelieff, who claims to have found traces of acetone in one case of nervous dyspepsia, while negative results were obtained in all other diseases of the stomach. I have repeatedly been able to demonstrate the presence of acetone in cases of carcinoma, and never have found it in neurotic conditions.

In order to test for acetone, the gastric contents are distilled after the previous addition of a small amount of phosphoric acid (1 to 1000), when the tests of Reynolds and Gunning (see Urine) are applied to the distillate. If both reactions furnish a positive result the presence of acetone may be regarded as demonstrated. Denigès' test may also be employed, and can be applied to the filtered contents directly (see Urine).

Vomited Material.

Food Material.—The vomiting of large amounts of totally undigested meat two or three hours after its ingestion is met with only in conditions associated with an entire absence of digestive juices from the stomach—*i. e.*, in cases of atrophic cirrhosis of the stomach (anadeny of Ewald). This condition is not to be confounded with the regurgitation of undigested food, mixed with mucus and saliva, which is seen in cases of stricture of the esophagus or of the cardiac orifice of the stomach. While at the outset of the latter disease the regurgitation of food occurs immediately, or at least very soon, after a meal, it may take place between meals in the later stages of the disease when dilatation has occurred. The recognition of the origin of the material brought up may then be exceedingly difficult. In such cases an examination should be made for biliary coloring matter, which, if present, will, of course, immediately exclude the esophagus as the source of the material ejected. Unfortunately, however, the reverse does not hold good. Small amounts of undigested meat are of no significance. The vomiting of well-digested food is observed in some of the neuroses of the stomach, and also in certain cases of acute and subacute gastritis, ulcer of the stomach, and chronic gastritis in its early stages. The vomiting referable to cerebral and spinal diseases also belongs to this category. In this connection it is very important to enquire into the existence of nausea previous to the vomiting, for, as is well known, considerable amounts of saliva and mucus may be swallowed if much nausea has existed, the result being that the process of digestion is arrested before the occurrence of vomiting. In such an event it would be erroneous to conclude that, because the material ingested has not reached that stage of digestion which would be expected at the time of the vomiting, the stomach is incapable of properly performing its functions.

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[1] Zeit. f. klin. Med., 1891, vol. xix, p. 19.

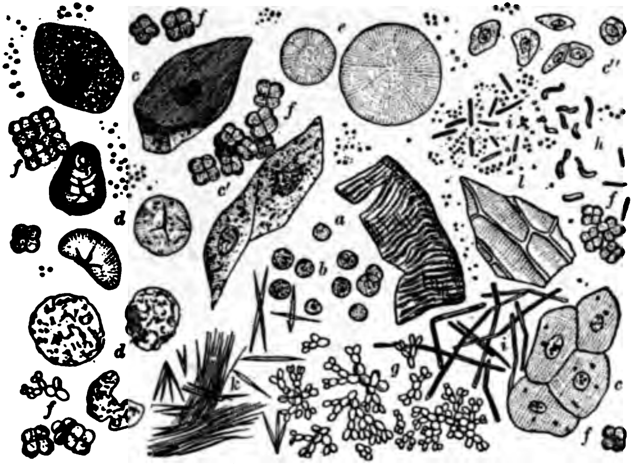

FIG. 68.—Collective view of vomited matter. (Eye-piece III, objective 8 A, Reichert.) *a*, muscle fibers; *b*, white blood corpuscles; *c*, *c'*, squamous epithelium; *c''*, columnar epithelium; *d*, starch grains, mostly changed by the action of the digestive juices; *e*, fat globules; *f*, sarcinæ ventriculi; *g*, yeast fungi; *h*, forms resembling the comma bacillus found by the author once in the vomit of intestinal obstruction; *i*, various microörganisms, such as bacilli and micrococci; *k*, fat needles, between them connective tissue derived from the food; *l*, vegetable cells. (v. Jaksch.)

Mucus.—The constant presence of large amounts of mucus in the gastric contents obtained with the stomach tube is almost pathognomonic of the mucous form of gastritis, while its presence in vomited matter may be referable to preëxisting nausea. In cases of pharyngitis moderate amounts of mucus are frequently found. The vomiting of pure mucus, according to Boas, is always pathognomonic of the absence of dilatation of the stomach, a statement founded on reason, as it is altogether unlikely that no particles of food should be brought up at the same time.

Under the term *gastrosuccorrhea mucosa* Dauber[1] has described a condition in which large amounts of mucus are secreted by the non-digesting organ, in the absence of symptoms pointing to a gastritis. I have observed a similar case occurring in a neurasthenic patient, in which enormous quantities of mucus could at times be obtained from the fasting organ, but never during the process of digestion. A mild degree of hyperchlorhydria existed at the same time, as well as enteritis mucosa and rhinitis mucosa. The motor power was practically normal.

Mucus is readily recognized on simple inspection by its glossy appearance. Chemically, it is distinguished by its behavior toward acetic acid (see Urine).

Saliva.—The vomiting of pure saliva in the morning upon rising is a fairly common symptom of chronic pharyngitis, which in turn frequently carries in its train a chronic gastritis; it constitutes the so-called *vomitus matutinus*. Saliva, like mucus, is, of course, always present in the gastric contents in small amounts. Larger amounts are usually referable to an increased secretion owing to the existence of nausea. Chemically, saliva is best recognized by testing for the presence of the sulphocyanides (see Saliva).

Bile.—Bile is rarely observed in the gastric contents brought up by the stomach tube, but is frequently seen in vomited matter, of which it may be said to be a constant constituent whenever the vomiting has been very intense or frequently repeated. Its presence in the former case should always excite suspicion of the existence of stenosis of the descending or horizontal portion of the duodenum or the beginning of the jejunum. This diagnosis becomes the more probable the more constant its presence.

Pancreatic Juice.—Mixed with the bile there is probably always present some pancreatic juice, and it has been suggested that the constant absence of this constituent, in the presence of bile, is strongly suggestive of pancreatic disease or of obstruction of the pancreatic duct (the ductus Wirsungianus).

The demonstration of pancreatic juice in the stomach is possible only if the reaction is neutral or alkaline, as the pancreatic trypsin is destroyed by pepsin-hydrochloric acid. If then hydrochloric acid is absent it is well to ensure a distinctly alkaline reaction by adding a little 1 per cent. solution of sodium carbonate; a flake of fibrin is added and the mixture placed in the incubator; if digestion takes place the presence of trypsin is established. The flakes of fibrin may be previously colored with a little Magdala red; as digestion takes place the red is liberated and colors the fluid.

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[1] "Ueber kontinuirliche Magen-Schleimsecretion," Arch. f. Verdauungskrank., vol. ii, p. 167.

Blood.—The presence of unaltered blood in the gastric contents is usually recognized without difficulty. If the hemorrhage has taken place in the stomach the color usually is dark brown or black owing to the action of the gastric juice upon the hemoglobin. Blood that is bright red in color and frothy is generally referable to a pulmonary hemorrhage, but it may happen that such blood remains in the stomach for some time and may then also appear brown or black. In the event of a large gastric hemorrhage, on the other hand, the blood may be vomited bright red in appearance.

In order to recognize mere traces when the macroscopic and even the microscopic examination do not point to the presence of blood, the method of Müller and Weber or that of Donogany should be employed. Kuttner claims that he was thus able to demonstrate the presence of blood in numerous cases of chlorosis in which other tests furnished negative results. I have been less successful in the disease in question, but admit that in cases of carcinoma and ulcer of the stomach it is with this method often possible to find traces of blood which would otherwise have remained unnoticed.

The recognition of such "occult" bleeding is at times of great value in diagnosis. (See Occult Blood in the Feces.)

Method of Müller and Weber.—The gastric contents are treated with a few cubic centimeters of strong acetic acid and extracted with ether. Should the ether not separate in a clear layer after a few minutes, a few drops of alcohol are added. If the ether then remains colorless, no blood pigment is present, while a brownish-red color indicates the presence of acetate of hematin. As a similar but yellowish-brown and much less intense discoloration of the ether may be produced by other pigments, such as biliary coloring matter, it is well, in doubtful cases, to test the ethereal extract with guaiacum or aloin. (See Tests for Occult Blood in the Feces.) Spectroscopic examination of the ethereal extract may also be resorted to. In the presence of blood an absorption band will be observed at the junction of the red and yellow.

Donogany's Method.—A small amount of the suspected material is extracted with a 20 per cent. solution of sodium hydrate and filtered. A drop of the filtrate is then mixed on a slide with a drop of pyridin and covered with a cover-glass, when, in the presence of blood, orange-red crystals of hemochromogen will separate out on standing for a few hours. On spectroscopic examination these crystals will show the characteristic band of absorption between the yellow and the green.

Hemorrhage from the stomach, *hematemesis*, may be observed in the most diverse conditions. It is either dependent upon a primary disease of the organ, such as ulcer and carcinoma, or it occurs secondarily to disease of other organs, leading to a hyperemic condition of the gastric mucosa, such as the various forms of cardiac, renal, and hepatic disease, in connection with menstrual abnormalities, etc. In melena, purpura hemorrhagica, pernicious anemia, etc., the cause of the hemorrhage cannot always be determined. Nervous influences also may take part in the causation of gastric hemorrhage.

Pus.—The occurrence of pus in vomited matter, referable to disease of the stomach itself, is uncommon. It is seen practically only in cases of phlegmonous and diphtheritic gastritis, and, as Strauss[1] has pointed out, in carcinoma affecting the smaller curvature and the region of the fundus. In such cases it is not uncommon to obtain as much as one-half to two tablespoonfuls of a mucopurulent fluid from the non-digesting organ. As the motor function in this form of carcinoma is often unimparied, the symptom may be of considerable value in diagnosis. The presence of larger quantities usually indicates perforation into the stomach of an accumulation of pus from a neighboring organ. An abscess of the liver, a suppurative pancreatitis, an abscess of the colon, or a subphrenic abscess may thus prove to be its primary source. When present in considerable amount pus is, of course, readily detected with the naked eye; if any doubt should arise, a microscopic examination will determine the question.

Stercoraceous Material.—Very important from a clinical standpoint is the vomiting of stercoraceous matter which is notably observed in cases of ileus. Usually this is recognized without difficulty by its odor, which is referable to the presence of skatol. If any doubt should arise, it is only necessary to distil the vomited matter after the addition of a little phosphoric acid, and to test for the presence of phenol, indol, and skatol in the distillate, as described in the chapter on Feces. When chiefly derived from the small intestine, the vomited matter, according to v. Jaksch, will contain bile acids and bile pigment together with an abundance of fat, which may be detected by chemical or microscopic examination. The reaction is usually alkaline or feebly acid.

I have had occasion to examine the vomited matter of a patient in whom an almost complete obstruction existed immediately above the ileocecal valve; the color of the material was a golden yellow, the reaction neutral; no bile pigment or biliary acids were found, while hydrobilirubin was present.

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[1] "Ueber Eiter im Magen," Berlin. klin. Woch., 1899, p. 870.

Parasites.—Of parasites, ascarides, segments of teniæ, trichinæ, Ankylostoma duodenale, and Oxyuris vermicularis are, at times, encountered. Protozoa have been described in the stomach contents of patients with carcinoma, by Hensen, Strübe, Zabel, Ullmann, Cohnheim, Nichols, and others. (See Microscopic Examination of Stomach Contents.)

Odor.—The odor of normal gastric juice is peculiar, suggesting the presence of an acid, which can be sharply distinguished from acetic or butyric acid. If blood is present in large amount, the vomitus emits an odor which is perfectly characteristic. A feculent odor is met with in cases of enterostenosis or in the presence of an abnormal communication between the stomach and the small or large intestine. A putrid odor may be observed in cases of ulcerative carcinoma, pyloric stenosis referable to ulcer, simple carcinoma of the stomach, muscular hypertrophy of the pylorus, stenosis due to inflammatory adhesions, etc. In cases of phosphorus poisoning the vomited matter emits an odor of garlic; the odor observed in uremic conditions is referable to ammonia; a carbolic acid odor is met with in cases of poisoning with this substance.

MICROSCOPIC EXAMINATION OF THE GASTRIC CONTENTS.

In the gastric contents obtained from the non-digesting stomach the various morphological constituents of mucus and saliva, which have been described elsewhere, are found. Microscopic particles of food, such as elastic tissue fibers, starch granules, fat droplets, fatty acid crystals, vegetable and muscle fibers, are, furthermore, quite constantly seen. Leukocytes and isolated nuclei also are observed; the latter are set free by the action of the gastric juice upon the mucous corpuscles and epithelial cells.

If gastric juice is allowed to stand, small tapioca-like bodies will collect at the bottom of the vessel, which upon microscopic examination will be seen to contain numerous snail-shell-like formations, occurring either singly or collected in groups. These probably consist of altered mucin, as they can be produced artificially by adding a sufficient amount of dilute hydrochloric acid to saliva. According to Boas, they are of no diagnostic significance.

Epithelial cells, fragments of the epithelial lining of the ducts of glands, as well as goblet cells, are not infrequently met with in the juice obtained from the non-digesting organ. In addition, various microörganisms, such as the Leptothrix buccalis, Bacillus subtilis, saccharomyces, micrococci (often arranged in the form of tetrahedra), Clostridium butyricum, etc., may be encountered.

Among the bacteria which may be found in the gastric contents under pathological conditions the bacillus described by Boas and

Oppler[1] is undoubtedly the most important, and has attracted much attention. It is quite constantly present in carcinoma, at a time when lactic acid can be demonstrated in large amount. It is an active lactic acid producer and its presence may hence be regarded as indicating advanced lactic acid fermentation. It is almost always absent in non-malignant disease of the stomach. The organism (Fig. 69) is non-motile, and essentially characterized by its great length and by the fact that the individual bacilli are frequently seen joined end to end, forming long threads and zigzag lines. Often the entire field of vision is filled with dense conglomerations, and in advanced cases it is usual to find the Boas-Oppler bacillus present almost exclusively in viable form. The organism is readily stained with the usual aniline dyes. I have succeeded in growing the organism on blood serum and usually also on plain agar, but it is very apt to undergo changes in size which may lead one to think that it has been lost or overgrown by other bacilli. Growth may sometimes be obtained by rendering the culture medium acid with lactic acid to the extent to which this was present in the stomach contents.

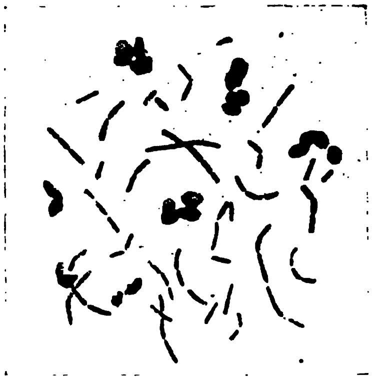

FIG. 69.--Boas-Oppler bacillus.

Tubercle bacilli may be found in vomited matter in cases of phthisis, where the sputa have been swallowed. Tuberculous ulceration of the stomach is exceedingly rare. Simmonds reports that in 2000 autopsies of tuberculous individuals the condition was noted only eight times.

Sarcinæ (see Fig. 68) occur in the form of peculiar colonies of cocci, arranged in squares or tetrahedra, resembling cotton bales. Not infrequently they are encountered under normal conditions, but only in small numbers. In pathological conditions, on the other hand, a drop of the gastric contents may constitute an almost pure culture. A case is on record in which the pylorus had become entirely occluded by an inspissated mass of these organisms. Whenever present the existence of certain fermentative processes may be inferred. It is curious to note that in advanced cases of carcinoma of the stomach sarcinæ are practically never seen, although the conditions are apparently most favorable for their development. Oppler[1] was unable to find them twenty-four hours after their introduction in large numbers and in pure culture. In cases of carcinoma of the curvatures and the walls, as also in advanced pyloric carcinoma, sarcinæ were never found, while they may be present in incipient cases of pyloric carcinoma so long as hydrochloric acid is secreted.

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[1] "Zur Kenntniss des Mageninhalts bei Carcinoma ventriculi," Deutsch. med. Woch., 1895, Nr. 5. Kauffmann, "Ueber einen neuen Milchsäurebacillus," etc., Wien. klin. Woch., 1895, Nr. 8. Schlesinger u. Kauffmann, Wien. klin. Rundschau, 1895, Nr. 15.

Protozoa have been found in the stomach contents by several observers. Nichols[2] has collected 23 cases from the literature. The most common are trichomonads and next in order Megastoma entericum (Lamblia intestinalis); whether or not still other varieties occur is not clear from the meager descriptions which are usually given. Flagellates, amebas, and monads are mentioned in a general way. Megastoma and trichomonads may be found together. The presence of protozoa is most common in carcinoma of the stomach (19 out of 23 cases). The reaction of the material in which they are found is almost invariably alkaline or neutral. It is noteworthy that in several cases trichomonads were also found in carious teeth and in many in the stools of the patients.

In esophageal carcinoma protozoa have also been found in the esophageal material.

From the available data there can be no question that the presence of protozoa in the stomach contents is suggestive of non-obstructive carcinoma. To hunt for the parasites it is best to obtain material from the fasting organ and to examine this as soon as possible, taking care that it is not exposed to cold. Attention should be especially directed to any solid particles that may be visible with the naked eye.

In vomited material containing biliary coloring matter, leucin, tyrosin, and cholesterin are quite commonly observed, and may be recognized by the form of their crystals, as well as by their chemical reactions, which are described elsewhere.

The occurrence of blood and pus in the gastric contents has been considered.

It not infrequently happens that small shreds of mucous membrane are brought away by the stomach tube, and in cases of chronic gastritis, hyperchlorhydria not dependent upon ulcer, and in some of the neuroses, this is indeed not at all uncommon.[3] Boas even suggests that in the neuroses, where fragments of mucous membrane are so readily detached, this may possibly be connected etiologically with the formation of ulcers, and there can be no doubt that the mere action of the abdominal muscles exerted during the process of defecation may be sufficient to detach such fragments. From the microscopic appearance of the particles the diagnosis between a gastric neurosis and one of the various forms of chronic gastritis may frequently be made, and the same may be said to hold good in the differential diagnosis between a true gastritis and a glandular insufficiency referable to passive congestion of the gastric mucosa.

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[1] Münch. med. Woch., 1894, No. 29.

[2] Amer. Journ., July, 1905, p. 120. G. Strübe, "Trichomonas hominis bei Carcinoma ventriculi," Berlin. klin. Woch., 1898, p. 708. P. Cohnheim, Deutsch. med. Woch., 1903, vol. xxix, p. 206.

[3] M. Einhorn, Med. Record, June 23, 1894; Berlin. klin. Woch., 1895, No. 20; Arch. f. Verdauungskrankheiten, vol. v, Heft 3.

At times *tumor particles* also are found in the gastric contents.[1] When particles of tissue are found they should be hardened at once, and then sectioned.

EXAMINATION OF THE MOTOR POWER OF THE STOMACH.

Under physiological conditions the stomach should contain but few particles of food, or none at all, six hours after the ingestion of Riegel's meal, or one and one-half to one and three-quarters hours after that of Ewald. A delay in the propulsion of the gastric contents may be referable to the existence of a simple atony or to dilatation of the stomach. According to Boas, an atony may usually be diagnosticated if, following the exhibition of a supper consisting of bread and butter, cold meat, and a large cupful of tea, the stomach is found empty in the morning, providing, of course, that symptoms exist which point to atony or dilatation. It should be remembered, however, that in cases of acute and subacute gastritis, in the absence of a more serious lesion, food may be found in the stomach twenty-four hours after its ingestion. A dilatation may, on the other hand, be diagnosticated if the stomach under the same conditions contains a considerable amount of food. In such cases it happens that not only remnants of the test supper, but remains of meals taken one, two, three, or even more days previously are found. The quantities, moreover, which may be obtained at the time of examination are often surprisingly great, and may amount to sixteen pounds or more. Portel cites the case of the Duc de Chausnes, one of Paris' greatest gourmands, whose stomach could hold 4.5 liters—*i. e.*, 8 pints.

The following methods may be employed for the purpose of testing the motor power of the stomach:

Leube's Method.[2]—Six hours after the ingestion of Riegel's meal the stomach is washed out with about 1000 c.c. of water. In the presence of only slight traces of food the motor power may be regarded as normal. This method is undoubtedly the most convenient for practical purposes.

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[1] P. Cohnheim, "D. Bedeutung kleiner Schleimhautstückchen f. d. Diagnostik d. Magenkrankheiten," Arch. f. Verdauungskrankheiten, 1896, vol. i, p. 274.

[2] Deutsch. Arch. f. klin. Med., vol. xxxiii.

The Salol Test of Ewald and Sievers.[1]—This test is based upon the observation that salol is decomposed into phenol and salicylic acid only in an alkaline medium. As the salicylic acid is eliminated in the urine as salicyluric acid, it is possible to determine the time of the passage of the salol from the stomach to the small intestine.

A capsule containing 1 gram of salol is given to the patient immediately after his breakfast or dinner, when separate portions of urine, passed one-half, one hour, two hours, and twenty-four hours later, are tested by adding a small amount of a solution of ferric chloride. In the presence of salicyluric acid a violet color results. Under normal conditions a positive reaction is obtained after from forty-five to seventy-five minutes. A further delay may usually be regarded as indicating the existence of motor insufficiency. If no result is obtained after twenty-four hours, a pyloric stenosis undoubtedly exists. Under normal conditions, furthermore, it will be observed that the salol elimination is completed after twenty-four hours, while in cases of dilatation of the stomach a positive reaction may still be obtained after thirty hours. It is thus possible to distinguish between dilatation and descent of the stomach.

The test, while it is convenient and usually yields fair results, is not altogether reliable, as the decomposition of the salol may at times occur in the stomach, owing to the presence of alkaline mucus, or may be delayed in the intestines owing to the existence of acid fermentation, etc.[2]

EXAMINATION OF THE RESORPTIVE POWER OF THE STOMACH.

To this end a capsule containing 0.2 gram of potassium iodide is given to the patient shortly before a meal, and the saliva examined for the presence of potassium iodide at intervals of from two to three minutes.[3] To this end strips of filter paper moistened with starch solution are immersed in the saliva, which has been acidified with nitric acid; the paper turns blue if iodide be present. Under normal conditions a violet color is obtained after from six and one-half to eleven minutes, and a bluish tint after from seven and one-half to fifteen minutes. In pathological conditions a delayed reaction is observed in almost all diseases of the stomach, and is especially marked in cases of dilatation and carcinoma, less so in chronic gastritis, and variable in ulcer.

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[1] Therap. Monats., August, 1887.

[2] Brunner, Deutsch. med. Woch., 1889. Huber, Correspondenzbl. f. schweizer Aerzte, 1890.

[3] Penzoldt, Berlin. klin. Woch., 1892 Faber, Inaug. Diss., Erlangen, 1882.

Absolute conclusions, however, cannot be drawn from results thus obtained, as a normal reaction time has also been observed in cases of dilatation and chronic gastritis.

INDIRECT EXAMINATION OF THE GASTRIC JUICE.

Günzburg's Method.—In those cases in which for any reason the introduction of the stomach tube is contra-indicated or impracticable the following method, suggested by Günzburg, may be employed:

A tablet of 0.2 to 0.3 gram of potassium iodide is inserted into a piece of the thinnest possible, strongly vulcanized rubber tubing, measuring about 2.5 cm. in length. The ends are folded as shown in Fig. 70, and the little package tied with three threads of fibrin hardened in alcohol. Every package should be examined before use, by immersion in warm water for several hours, to determine its tightness, testing for the presence of potassium iodide by means of starch paper and fuming nitric acid. One of these packages is swallowed by the patient three-quarters to one hour after an Ewald test breakfast, and the saliva tested for potassium iodide at intervals of fifteen minutes, until a positive result is reached or until six hours have elapsed. It is unnecessary to wait longer than six hours. In the presence of free hydrochloric acid the threads of fibrin are dissolved and the potassium iodide absorbed. Under normal conditions a positive reaction is obtained after from one to one and three-quarters hours, while anachlorhydria undoubtedly exists if no result is obtained within five or six hours. In cases of hypochlorhydria the reaction is delayed for more than two to three hours. Günzburg further advises that the resorption test with potassium iodide be also made, and that the reaction time be deducted from that taken up in the elimination of the iodide contained in the package. Several tests, moreover, should be made in the same case.

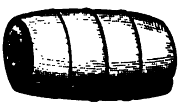

FIG. 70.—A fibrin-potassium-iodide package of Günzburg.

I have had occasion to experiment with packages obtained from Germany, and manufactured according to the directions of Günzburg.[1] In most of the packages the threads of fibrin had become brittle and were broken in transit. The results obtained with about twenty intact specimens, however, were entirely satisfactory, and it is to be regretted that the packages cannot be obtained in the American market.

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[1] Göthe Apotheke, Frankfurt a. M.

Similar packages have been constructed by Sahli (*desmoid reaction*). In this case pills of methylene blue or iodoform are enclosed in little pieces of rubber tissue and closed with catgut. They are administered at the noon meal and the urine (viz., saliva) tested at 5 and 7 P.M. and again in the morning.[1]

Reach has of late made use of barium iodate and the oxyiodate of bismuth for the same purpose, but without enclosing the substance in rubber. As hydrochloric acid only is capable of liberating the iodine from these bodies, they may be employed instead of the Günzburg packages. As a result of his examinations, he concludes that in the presence of hydrochloric acid iodine can thus be demonstrated in the saliva within eighty minutes. He finds, however, that at times the reaction occurs later than might have been supposed from the amount of hydrochloric acid found.

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[1] Monod, Journ. de physiol. et de pathol. gén., 1906, vol. viii, p. 853.

CHAPTER IV.

THE FECES.

THE feces constitute a mixture of indigestible and undigested particles of food, of unabsorbed secretions of the gastro-intestinal tract, and their decomposition products, together with intestinal mucus, epithelial cells, and bacteria.

EXAMINATION OF NORMAL FECES.

General Characteristics.

Number of Stools.—The number of stools which may be passed in the twenty-four hours is subject to wide variation, even under physiological conditions, but is usually constant for one and the same individual. One or two stools *pro die* may be regarded as normal. Exceptions, however, are frequent. Persons are thus met with who have but one stool every two to four days, and cases are on record in which only one passage occurred every seven to fourteen days, the individuals evidently enjoying perfect health. On the other hand, the number of stools may be increased to three or four under strictly normal conditions. *Hence the importance of accurately ascertaining the habitual number of stools in every individual.* It would thus be manifestly wrong to regard the passage of three stools daily as diarrhea, or the passage of only one stool in forty-eight hours as constipation, if this number has been habitual throughout life.

Diarrhea is said to exist when the consistence of the stools is materially diminished; the number is then also usually increased. This may vary from two to thirty, forty, and even fifty in the twenty-four hours. On the other hand, a single stool in the twenty-four hours may constitute diarrhea. The most extreme grades of diarrhea are observed in Asiatic cholera, dysentery, and the summer diarrhea of infants.

Amount.—In those cases in which more than one or two stools occur in twenty-four hours it is well to ascertain the amount actually passed. The normal amount varies between 100 and 200 grams.[1] This quantity is increased by a diet rich in vegetable and starchy foods, and is diminished by one rich in animal proteids, so that 60 and 270 grams may be regarded as the extreme limits in health. Such amounts as 500 and 1000 grams are certainly abnormal.

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[1] Voit, Zeit. f. Biol., vol. xxv, p. 264.

Average quantities for various ages are given in the following table, which is taken from Schmidt and Strassburger:[1]

Age.	Diet.	Average amount of feces in twenty-four hours.
Child, 1 month old	Mother's milk	3.3 grams
" 2 to 3 months old . .	" "	6.5 "
" 7 " " . . .	Variable	15–56 "
" 9 " " . . .	Cows' milk with additions	59.0 "
" ¾ to 2 years old. . . .	Mixed	77.0 "
" 4 " "	"	101.0 "
" 6 " "	"	134.0 "
" 9 " "	"	117.0 "
" 11 " "	"	138.0 "
Adult	"	131.0 "

Unusually large amounts of fecal matter may be observed following an attack of constipation of long duration or an attack of obstruction. Lynch reports a remarkable instance in which, following a prolonged attack of constipation, an enema caused the evacuation of 20 kgrms. of fecal matter. Especially large amounts of feces are observed in cases of biliary obstruction, where 1100 grams may be exceeded. In cases of fermentative dyspepsia the amount may also be large, varying between 400 and 900 grams, while the patients are on a diet on which normal individuals would pass from 200 to 270 grams in the twenty-four hours. Still larger amounts are noted in cases of enteritis. Schmidt mentions a case in which 2780 grams were eliminated (these figures have reference to a three days' experiment with a test diet; see p. 268).

Consistence and Form.—The consistence of a stool depends essentially upon the amount of water present, and hence upon the nature of the food ingested, being softer with a purely vegetable diet (80 to 85 per cent. of water) than with a diet rich in animal proteids (60 to 65 per cent.). With a mixed diet the amount of water corresponds to about 75 per cent. As a general rule, normal stools exhibit the characteristic cylindrical form and are fairly firm. Mushy stools, however, are also seen quite frequently, and round, scybalous masses, although far more common in constipation, may likewise be observed in health. The individual scybala usually vary in size from that of a hazelnut to that of a walnut, and are frequently provided with one or two indentations which represent impressions of the tenia of the colon. Still smaller masses, resembling the dejecta of sheep, may also be seen. Their presence was formerly regarded as characteristic of stricture of the colon, but they are likewise found in ordinary cases of chronic constipation. Fecal ribbons and columns of the diameter of a pencil are found in cases of enterospasm of neurotic origin, as well as in stricture of the colon.

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[1] Die Faeces d. Menschen, Berlin, 1961, A. Hirschwald.

Odor.—The repugnant odor of the feces is, to a large extent, due to the presence of indol and skatol and in some cases also to hydrogen sulphide, methane, and phosphine. A most disagreeable odor is met with in the so-called acholic stools. The odor of fatty acids is observed in the lighter grades of infantile diarrhea, while a markedly putrid odor is associated with its severer forms. A very characteristic, sperm-like odor is noted in the stools of cholera, owing to the presence of considerable quantities of cadaverin. A truly rotten stench is present in the gangrenous form of dysentery, and in carcinomatous and syphilitic ulceration of the rectum. An ammoniacal odor is due to an admixture of urine undergoing ammoniacal decomposition.

Color.—The color of the feces varies, according to the nature of the food ingested, from a light to almost a blackish brown, a firm stool being in general darker than a thin stool. A stool that has remained exposed to the air is also somewhat darker upon its outer surface than in its interior, owing to processes of oxidation. In nursing infants, in consequence of the exclusive ingestion of milk, the color is light yellow.

Under normal conditions the color is never due to native biliary coloring matter, but is largely dependent upon the presence of urobilin. It is, furthermore, influenced by the nature of the food, chlorophyll tending to produce a greenish color, starches a yellowish tinge. If much blood is present in the food the feces may be almost black, owing to the formation of hematin. Huckleberries and red wine likewise produce a blackish color, chocolate and cocoa a gray; preparations of iron, manganese, and bismuth color the feces dark brown or black, owing to the formation of sulphides of these metals; the green color of calomel stools was formerly supposed to be due to the formation of a sulphide, but is more likely caused by the presence of biliverdin. Santonin, rheum, and senna produce a yellow color. Quite characteristic also are the ipecacuanha stools, which closely resemble the so-called acholic stools.

The color of the feces in disease may vary a great deal. When unaltered bile is present, the stools may assume a golden-yellow, a greenish-yellow, or even a green color. In cases of biliary obstruction or suppression, on the other hand, they become pasty and have a grayish or even a white color. This, however, is not so much due to the absence of coloring matter derived from the bile as to an insufficient absorption of fats, as was shown by Strümpell, who succeeded in obtaining stools of a light-brown color after feeding patients affected with catarrhal jaundice upon a diet containing minimal amounts of fat. *Such acholic or colorless stools*, as it would be better to say, are not only found associated with biliary obstruction, but may also occur when the ducts are patent. They have been observed in various cases of leukemia, carcinoma of the stomach or intestine, in simple infantile enteritis, chronic nephritis, chlorosis, scarlatina, tuberculous enteritis, and especially frequently in debilitated consumptives and in cases of chronic tuberculous peritonitis in children. In some of these conditions, as in tuberculosis of the intestines and of the peritoneum, the lack of color is probably due to a diminished absorption of fats. In others, however, this explanation does not hold good, as abnormally large amounts of fat are not necessarily present. In such cases the lack of color is probably referable to the formation of colorless decomposition products of bilirubin, such as the leuko-urobilin of Nencki. In this connection it may be interesting to note that in those cases in which the biliary ducts are patent the color of the stools may vary not only from day to day, but even within the twenty-four hours. A neurasthenic patient occurring in my practice thus passed an acholic stool almost every morning and usually colored feces in the afternoon, for a period of several weeks.

Generally speaking, the color of the stools becomes lighter the larger the number of movements, and *vice versa.* In Asiatic cholera and dysentery they may be colorless, while in severe constipation the scybalous masses are almost black.

An admixture of *pus* in notable amounts also gives rise to a characteristic color, as is seen in cases of dysentery, syphilitic and carcinomatous ulceration of the colon and rectum, following the perforation of a parametritic or periproctitic abscess into the rectum, etc.

Carter and MacMunn[1] have recently pointed out that at times a chromogen may be present in the feces, which on exposure to the air is transformed into a red pigment, simulating blood-coloring matter. They report three cases in which this was observed. MacMunn expresses the opinion that the substance in question is closely related to stercobilin. The stools showed streaks of red upon the surface, and after further exposure and repeated agitation turned a pronounced blood red throughout.

Green stools are observed especially in infants, and may be referable to two different causes, being dependent on the one hand upon the presence of a bacillus, described by Le Sage, which produces a green coloring matter, while on the other it may be referable to biliverdin. When green stools occur frequently, this condition is associated with the clinical symptoms of a severe cholera infantum. Such stools have also been noted in dysentery referable to infection with the Bacillus pyocyaneus.

If *blood* is present the stools may present a scarlet red, a dirty brownish red, a coffee, or even a perfectly black color. *Adherent blood,* usually bright red in color and found on scybalous masses, is probably always derived from the rectum or anus, while a change in color, indicating an earlier date of the bleeding, usually points to the colon.

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[1] Brit. Med. Jour., 1899.

An *intimate admixture of blood* with the stool, the color being at the same time altered, so as to vary from a brownish red to black (owing to the presence of ferrous sulphide), is indicative of hemorrhage into the stomach or the small intestine. The darker the color the more remote from the anus will be, as a rule, the seat of the hemorrhage. Black or coffee-colored stools are thus observed in cases of ulcer of the stomach or of the duodenum, in melena neonatorum, and similar conditions.

When profuse intestinal hemorrhages take place, however, as in some cases of typhoid fever and melena, and particularly when diarrhea exists at the same time, the blood which appears in the stools may be changed very little or not at all.

While simple inspection or a microscopic examination of the feces will often determine whether or not blood is present, it has been ascertained that *occult* bleeding may frequently occur where the presence of blood can only be established by special chemical examination. Evidence of such occult bleeding can be obtained in malignant growths involving the gastro-intestinal tract, in ulcus (over 80 per cent. of the cases), hemorrhagic pancreatitis, catarrhal jaundice (at the height of the disease), general venous stasis referable to heart lesion. Other sources of bleeding must of course be excluded, and the diet during the period of examination should be free from meats.[1] The aloin test is best employed.

Aloin Test.—If the stools are not in a semiliquid condition they must be made so by thoroughly mixing them with distilled water; 5 grams of stool are usually sufficient. The material is then extracted by shaking with an equal volume of ether. The mixture is allowed to stand for fifteen minutes or longer and the supernatant fluid poured off. The remaining fecal material is mixed with one-third its volume of glacial acetic acid and 10 c.c. of ether. The mixture is again thoroughly shaken and set aside for the ethereal layer to separate out, and this then poured off.

The aloin solution which is now used is prepared by dissolving as much aloin as will go on the end of a spatula in one-third of a test tube of 70 per cent. alcohol; 2 to 3 c.c. of the clear yellow solution are mixed in a test-tube with about the same amount of the acetic ethereal extract and treated with 2 to 3 c.c. of ozonized turpentine (prepared by allowing chemically pure turpentine, such as that of Merck, to stand exposed to the air for at least three weeks), or an equal amount of active hydrogen peroxide. The mixture is thoroughly shaken. If blood is present the reaction may appear in one of several ways: either the whole mixture turns pink, which gradually deepens to a cherry red, or the solution of aloin sinks to the bottom and forms a layer beneath the mixture of ether and turpentine, and this lower layer of aloin in positive tests gradually becomes a deep cherry red. Sometimes if the ether and turpentine are first mixed and the aloin is then allowed to flow gently down the side of the tube, the two sets of fluid will remain separate and a deep-red ring will form at their junction. Not more than fifteen minutes should be allowed for the red color to show itself, for after this the aloin will gradually turn red even if blood is not present. It is necessary to make the aloin solution freshly, for when it stands exposed to the light it changes to about the color that it attains in the reaction when blood is present.

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[1] Steele and Butt, Amer. Journ., July, 1905, p. 36. Hartmann, Arch. f. Verdauungsk., 1904, vol. x, Heft 1. Joachim, Berlin. klin. Woch., 1904, No. 18.

If the test is negative the color remains a light yellow, which becomes red after standing for some length of time.

Guaiac Test.—This test may also be employed, but is not quite so satisfactory as the one preceding. The ethereal extract of the fecal material is prepared as described. The reagent is made by shaking a gram or so of gum guaiac in a test-tube half-full of ether and allowing the mixture to stand until it becomes clear by settling. A couple of c.c. of this solution are added to the same amount of the ethereal extract of the feces and at least an equal volume of hydrogen dioxide is added. The whole is shaken; the hydrogen dioxide settles to the bottom and the ethereal extract floats on top. The blue color (owing to the oxidation of the guaiaconic acid to guaiac blue) of a positive reaction shows itself very quickly in the supernatant fluid, which in a decided reaction becomes a deep blue, that may be somewhat masked by the brown color of the urobilin in the ethereal extract. In such a case the blue color often becomes a purplish brown, but even this reaction is unmistakable. If the reaction is negative no color change occurs. The guaiac solution must be fresh, but need not be made up daily.

Macroscopic Constituents.

Alimentary Detritus.—Upon gross examination of the feces it is possible to find stones of cherries, grape seeds, woody vegetable fiber, the skins of berries, large pieces of connective tissue, undigested pieces of apple, pear, potato, grains of corn, etc.

The presence of notable amounts of digestible food, such as pieces of muscle tissue, flakes of casein, fragments of amylaceous food, constituting what was formerly spoken of as *lientery*, is always indicative of disturbed gastric or intestinal digestion. It is hence observed in chronic intestinal catarrh, febrile dyspepsia, etc. Occasionally also unaltered food in large amounts is found in the feces, owing to a direct communication between the stomach and the colon, as in cases of perforating ulcer or carcinoma of the stomach.

When fat is present in abnormally large amounts it can usually be recognized with the naked eye. To this condition the term *steatorrhea* has been applied. In typical cases the fat is seen in the form of whitish or grayish masses, varying in size from that of a pea to that of a walnut, which are more or less intimately mixed with the fecal material, and may at first sight be mistaken for flakes of casein. From these it may be distinguished by its chemical reactions and its peculiarly glistening appearance. In other cases stools may be seen in which the fecal column is covered, to a greater or less extent, with a grayish, dense, asbestos-like substance, while the core itself presents the usual color. Nothnagel states that this appearance is referable to congealment of the fat when it is exposed to a lower temperature than that of the body. I have repeatedly observed this appearance in stools which had just been voided and were still warm. In other cases the fat is intimately mixed with the feces, which are colored a light gray throughout. The passage of liquid oil in the absence of fecal material has also been recorded, but it seems doubtful that the oil in such cases entered the body by the mouth. Following the use of oil enemas such stools are, of course, seen.

The elimination of abnormally large quantities of fat may be due to the ingestion of correspondingly large amounts. More frequently, however, it is referable to pathological conditions. A steatorrhea will thus naturally occur when an insufficient supply of bile is poured into the small intestine, and hence is observed constantly in cases of biliary obstruction. True steatorrhea is also met with in diseases affecting the resorptive power of the small intestine, such as extensive atrophy or amyloid degeneration of the intestinal mucosa, tuberculous ulceration, etc., or in diseases involving the integrity of the lymphatic glands and vessels of the mesentery, as in chronic tuberculous peritonitis, caseous degeneration of the mesenteric glands, etc. In simple catarrhal conditions, however, steatorrhea may also occur, and not only in infants, but, according to my experience, also in adults. The question whether or not steatorrhea is constantly observed in cases of pancreatic disease, as some observers have claimed, may now be answered in the negative, although it must be admitted that the two conditions are very frequently associated. Le Nobel, who has investigated this subject, arrived at the conclusion that the steatorrhea in itself is of little practical importance, but that its association with the absence of products of putrefaction from the stools, the absence of the salts of the fatty acids, and the presence of maltose in the urine, may possibly be regarded as indicating the existence of pancreatic disease.

Mucus and Mucous Cylinders.—So long as mucus occurs in small particles only, adherent to otherwise normal feces, it is of no pathological significance. Larger amounts are almost always indicative of a catarrhal condition of the colon or rectum, no matter whether the stool is otherwise normal or whether diarrhea exists at the time. Peculiar formations are occasionally seen, viz., so-called *mucous cylinders*, which are passed in large or small fragments in a condition which has been described by Nothnagel as *enteritis membranosa* or *colica mucosa.*[1] Such masses, which at times measure a foot or more in length, are ribbon or net shaped, and are frequently passed in the absence of fecal matter, with severe tenesmus. They resemble Curschmann's spirals, but lack the central thread and the Charcot-Leyden crystals. They are probably indicative of chronic constipation associated with catarrh of the colon. Not to be confounded with this condition is the passage of masses of mucus, which do not present the cylindrical form, but which also may be passed with a great deal of tenesmus and in the absence of fecal matter; this is very commonly seen in cases of nephroptosis associated with gastroptosis and enteroptosis. These formations are in all probability also referable to a catarrhal condition of the colon. In cholera Asiatica particles of mucus are seen which resemble grains of rice; their presence was formerly regarded as characteristic of this disease, but they are now known to occur in ordinary catarrhal conditions also.

Biliary and Intestinal Concretions.—Most important from a diagnostic standpoint is the examination of the feces for the presence of biliary concretions, which should never be neglected in cases of colicky, abdominal pain of doubtful origin, whether associated with jaundice or not.

When searching for gallstones the feces should be stirred with water and passed through a fine sieve. Biliary concretions may then be found as small, crumbling masses, or as hard stones presenting an irregular contour or the smooth, characteristic facets. In size they may vary from that of a millet seed to that of a pigeon's egg; large stones are rarely passed by the bowel unless perforation has occurred into the intestines and usually into the colon.

Some calculi consist almost entirely of cholesterin, while others are composed essentially of inspissated bile, and still others of calcareous salts. The former are the most common, and are readily recognized by their softness and color, which may be white, grayish, bluish, or greenish. Their specific gravity is lower than that of water. Very frequently they contain a nucleus, composed of earthy sulphates or phosphates. An analysis which I made of a stone of this kind, weighing 10.548 grams, gave the following results:

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[1] Nothnagel, "Colica Mucosa," Beiträge z. Physiol. u. Path. d. Darmes, 1884. Fleiner, Berlin. klin. Woch., 1893, Nos. 3 and 4. Einhorn, Arch. f. Verdauungskrank., vol. iv, p. 456

Cholesterin	72.590 per cent
Mineral salts	0.247 "
Fats	5.090 "
Biliary pigments	13.930 "
Organic matter	7.270 "

Calculi which consist largely of biliary pigments are brown in color. They are hard, and heavier than water. Frequently they contain traces of copper and zinc (Fig. 71).

Calculi composed of calcareous salts generally present an irregular, roughened contour.

Welch has drawn attention to the not infrequent presence of pure colonies of the Bacillus coli communis in gallstones, apparently forming their nucleus. Typhoid bacilli also have since been observed in their interior, and it appears likely that the formation of gallstones is primarily referable to an invasion of the gall-bladder by such microörganisms. A remarkable case has been reported by Pearce, in which a leptothrix was the only microörganism found in biliary concretions, while in the bile this was present together with the colon bacillus.[1]

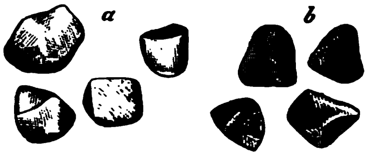

Fig. 71.—Gallstones: *a*, cholesterin; *b*, pigment stones.

Intestinal concretions (enteroliths) are rare and usually come from the appendix. At times they contain some foreign body, such as a grape seed, as a nucleus, upon which calcium and magnesium salts have become deposited.

Fecal calculi or *coproliths* are likewise only rarely seen. They represent inspissated fecal material which has become impregnated with lime and magnesium salts. More commonly they are found at the postmortem table in the cecum, in the haustra of the colon, and in the rectum.

Intestinal sand is also rare. I have seen only 5 cases in the past ten years. In the German literature I have found reports of only 3 cases, while in the French literature about 16 have been recorded. Of its origin nothing is known. The condition is commonly associated with enteritis membranacea. The material presents a brownish color, ut may be light green. In 1 case reported by Deetz[1] it was pos-ble to demonstrate the presence of calcium phosphate with traces of ilcium oxalate. One case is recorded by Thomson and Ferguson.[2] nalysis: 11.7 per cent. of $CaCO_3$; 87.3 per cent. of $Ca_3\ (PO_4)_2$; isoluble residue (silica), 1 per cent. There was present also a pig-ient which the writers regard as intermediary between ordinary bile igment and stercobilin. They think the sand is formed in the ileum.

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[1] Pearce, Univ. of Penna. Bull., Aug., 1901. Cushing "On the Presence of Typhoid Bacillus," Johns Hopkins Hospital Bull., 1899, p. 166; and Hunner, ibid., 1898, p. 163. Cushing, "On the Presence of the Colon Bacillus," ibid.; and Fournier, cit. by Chauffard, Rev. d. méd., 1897, p. 81.

Foreign Bodies.—In children, the insane, in cases of hysteria, nd even in people who are apparently possessed of their normal :nses, the physician must be prepared to find at times all kinds of reign bodies, such as pins, coins, buttons, false teeth, tooth-plates ith ragged edges, and even dirk-knives, all of which have been nown to pass through the alimentary canal. It must not be for-otten, however, that in cases of hysteria bodies may be shown by atients which they claim have passed by the rectum, but which ave been wilfully added to the stools, such as snakes, frogs, etc.

MICROSCOPIC EXAMINATION OF THE FECES.

General Technique.

The general technique in the microscopic examination of the eces is very simple. Stools that are firm when passed should be tirred up with water to a moderately thin mush. Drops of this iaterial are mounted on a series of slides, covered with cover-glasses, nd examined at first with a low power (⅔ B. & L.) and then with a iedium power (⅙ or ⅐). The survey with the low power furnishes general idea of the amount of food remnants (muscle fibers, frag-ients of vegetable material, fat), of the presence of crystals, pus, blood, nd eggs of parasites. The higher power (⅙ or ⅐) is reserved for gen-ral purposes of verification, to make out details of structure, and the earch for the smaller animal parasites (trichomonads, ameba coli, etc.)

If the stools are already thin when passed, no further dilution is iecessary. Bits of mucopus or of material showing the presence f blood are generally advantageous for the search for amebas. Ausgrave and Clegg, however, recommend that in doubtful cases t is well to administer a saline cathartic and to examine the fluid ortion of the resulting movements. In the examination for amebas it is essential that the stools be passed into a warmed bedpan and examined at once on warmed slides or by the aid of a warm stage. A convenient form of warm stage, which may be obtained from instrument-makers at low cost, is composed of brass and made to be held in position on the stage of the microscope by spring clips. It is about 8 cm. long and 3 cm. broad, and has cemented to a recessed bottom an ordinary glass slip; an opening measuring 1.35 cm. in diameter is in the centre of the stage. To one of the long slides of the brass stage is fitted a projecting stem, about 10 cm. long, to which the heat of a spirit-lamp is applied.

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[1] Deutsch. Arch. f. klin. Med., 1901, vol. lxx, p. 365. See also Dieulafoy, "La thiase intestinale et la gravelle de l'intestin," Presse méd., March 10, 1897 extract in Centralbl. f. klin. Med., 1897, p. 904).

[2] Jour. of Pathol. and Bact., vol. vi, 1900, p. 334. Laboulbène, Bull. Acad. de néd., Paris, 1873. Sheridan, Trans. Path. Soc. London, 1890, vol. xli, p. 111.). Thoma, Australasian Med. Gaz., Nov., 1891. Mathieu and Richaud, Soc. néd. d. hôp., May 22, 1896. S. J. Shattock, Trans. Path. Soc. London, vol. lviii.

Specimens containing eggs of parasites are readily preserved by the addition of 5 per cent. carbolic acid or of thymol.

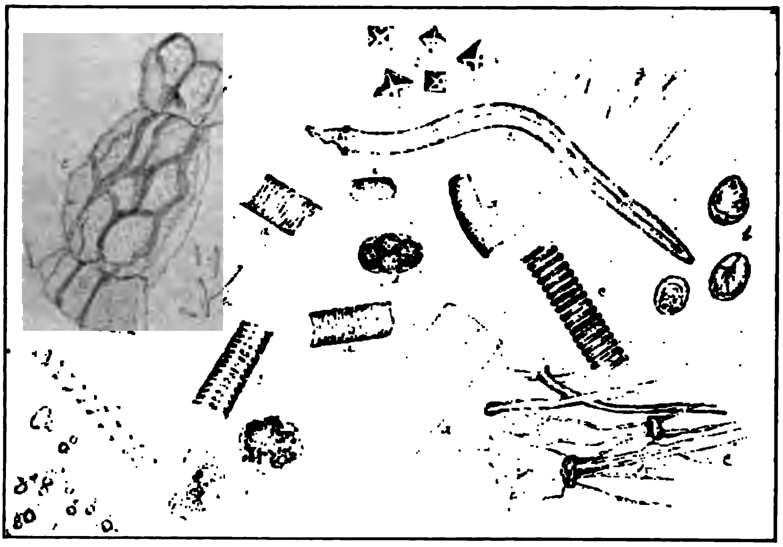

Fig. 72. Collective view of the feces: *a*, muscle fibers; *b*, starch granules; *c*, vegetable material; *d*, potato cells; *e*, egg of Uncinaria duodenalis; *f*, calcium oxalate crystals; *g*, fatty acid crystals; *h*, Charcot-Leyden crystals.

Unless living organisms are to be searched for, the stools if liquid may be placed in conical glasses and covered with a layer of ether so as to diminish the disagreeable odor; if mushy or firm they may be spread upon a plate and covered with a layer of turpentine.

Constituents Derived from Food.—Microscopically, indigestible and undigested constituents of food may be seen (Fig. 72), such as the framework of vegetable material, sometimes still containing starch granules or remnants of chlorophyll; muscle fibers, usually colored yellow and more or less altered in structure. Elastic-tissue fibers are readily recognized by their double contour and bold outlines. Connective-tissue fibers of the white variety can also generally be distinguished; when present in large quantities they are usually indicative of some digestive derangement, unless they are observed following the ingestion of a meal particularly rich in meat. Flakes of casein also are seen frequently.

Muscle fibers are found in every stool whenever meat has been eaten. Under normal conditions, however, they are not numerous, unless particulary large quantities have been ingested. Their appearance under the microscope may vary considerably. On the one hand, fibers are met with which still retain their characteristic features; others are split up either partially or entirely into the well-known disks; but more common than both are more or less roundish, yellow, apparently homogeneous fragments, which at first sight do not resemble muscle fibers in the least. Upon closer investigation, however, their true nature will become apparent. It will then be seen that two of the sides in some portions at least are more or less parallel, and if the specimen is examined with a high-power lens some traces of cross-striation can probably always be discovered.

Isolated starch granules are scarcely ever found under normal conditions, excepting in young children who have been fed with much starchy material. Starch granules enclosed in vegetable cells are likewise not found as a general rule, but are more common than the isolated granules. Their presence is easily recognized by treating microscopic preparations with a solution of iodopotassic iodide (Lugol's solution), when the granules or fragments will assume a blue color.

The presence of fat in the feces is quite constant, even in health. It may occur in the form of needle-like crystals, as fat droplets, or as polygonal masses which are highly refractive and often colored yellow or a yellowish red. Their true nature is easily recognized by adding a drop of concentrated sulphuric acid and heating, when they are transformed into the characteristic fat droplets.

The so-called *acholic stools* are usually very rich in fat, and particularly so in cases of biliary obstruction associated with jaundice. At other times the lack of color,. as has been mentioned above, is not referable to the secretion of an insufficient amount of bile, but to the presence of colorless decomposition products of bilirubin, such as the leuko-urobilin of Nencki. In these cases abnormally large quantities of fat are not always present. The conclusion that a stool contains excessive amounts of fat because it is apparently acholic is hence not justifiable unless a microscopic examination has been made.

In pathological conditions it is necessary to determine whether or not food remnants are present in abnormal amount, presupposing, of course, that excessive quantities have not been ingested. It is often possible to draw definite conclusions as to the state of intestinal digestion from the excess of one form of non-digested material over another. The presence of large quantities of undigested starch indicates a catarrhal condition of the small intestine, and it may, indeed, be said that the occurrence of more than traces of this material should be regarded with suspicion. An increase in the number of muscle fibers will, as a rule, likewise be observed under such conditions.[1]

Schmidt and Strassburger[2] have described a special form of intestinal fermentative dyspepsia, in which there is an isolated amylolytic insufficiency, which may be of functional or of organic origin. (See Schmidt's fermentation test below.)

In this connection it is noteworthy that in man extensive disease of the pancreas may exist without seriously disturbing amylolytic digestion.

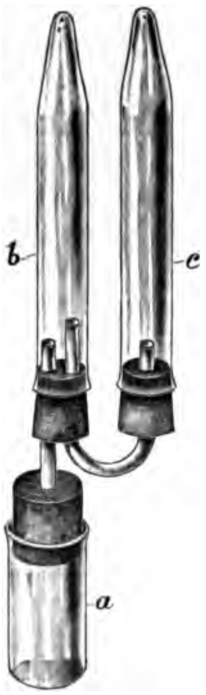

FIG. 73.—Schmidt's fermentation tubes.

Schmidt's Fermentation Test.—To obtain a more exact insight into the degree of amylolytic insufficiency of the intestinal tract than is possible from a microscopic study of the feces, Schmidt has proposed a special method which is based upon the continued digestion of the carbohydrates in the feces. The examination is made after the patient has been placed on the following test diet (Schmidt and Strassburger's test diet No. II): milk, 1.5 liters; 3½ eggs; strained oatmeal gruel (from 80 grams of oatmeal); 100 grams of Zwieback; 20 grams of butter; 20 grams of sugar; 125 grams of steak (raw weight), and 190 grams of potato (raw weight). The distribution of these various articles of food can be arranged as one chooses, or as follows: At 7.30 A.M., ⅜ liter of milk and 2 Zwiebacks (each 33 grams); at 10.30 A.M., ⅜ liter of bouillon with ½ egg; at 12 M. ⅜ liter of milk with 1 egg; between 1 and 2 P.M. ½ liter of oatmeal gruel (prepared from 40 grams of oatmeal, 166 grams of milk, 10 grams of sugar, and ½ egg); 100 grams of well-done Hamburg steak (125 grams of raw beef, raw weight) and 12 grams of butter; 250 grams of mashed potato (from 190 grams of potato, 60 grams of milk, and 8 grams of butter); at 4.30 P.M., ⅜ liter of milk, 1 egg, 1 Zwieback; at 7.30 P.M., ½ liter of oatmeal gruel as at dinner-time. Before commencing with the test diet, however, it is necessary to demarcate the fecal material by giving a wafer or capsule containing 0.3 gram of powdered carmine. The examination proper is made as soon as the feces are no longer colored red, viz., after from two to three days of the test diet. The necessary apparatus is pictured in the accompanying figure (Fig. 73), which represents one-third of the acutal size. For each experiment 5 grams of fresh fecal material are used (the feces being of medium consistence; otherwise a little more or less is taken, corresponding to about 1 gram of dry residue). The material is well stirred with water in the bottle *a*, which is filled entirely and closed with the rubber stopper, care being taken to exclude bubbles of air. Tube *b* is filled with water from the tap and also closed without admission of air. Tube *c* should contain no water; it has a pinhole aperture at the top. The communicating tube *d* is adjusted as shown in the figure. The apparatus is then placed in the incubator at 37° C. for twenty-four hours, not longer. During this time the carbohydrate fermentation will have been completed (Schmidt's Frühgährung). During the evolution of gas water will be displaced from *b* into *c;* the resulting column is measured and represents the degree of fermentation. The result is regarded as positive if more than a quarter tubeful of gas is obtained. With the test diet in question this would mean a condition approximating the normal. In such an event the patient is placed for two days further on test diet No. I, which differs from No. II only in the absence of the meat and potato. If then there is still a positive result, the diagnosis of "fermentative dyspepsia" is justifiable. In order to eliminate errors arising from possible formation of gas as the result of albuminous putrefaction the fermenting fecal material should be tested from time to time in a control specimen. If the formation of gas is due to carbohydrate fermentation, there will be an increasing degree of acidity (tested with litmus paper); this increase, however, is not always marked; at any rate, there must be no increasing alkalinity.

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[1] Schmidt u. Strassburger, Deutsch. Arch. f. klin. Med., vol. lxix, p. 570.

[2] "Die klinische Bedeutung der Ausscheidung von Fleischresten mit dem Stuhlgang," Deutsch. med. Woch., 1899, p. 811.

Leiner's Test for Casein.—Casein is most conveniently demonstrated with Leiner's method. To this end a small amount of fecal matter is spread on a slide and dried in the air. It is then fixed by heat—passing the specimen through the flame of a Bunsen burner three or four times is sufficient—and stained with a mixture of equal parts of a 0.75 per cent. solution of acid fuchsin and methyl green in 50 per cent. alcohol, the mixture being diluted ten times with water. After fifteen minutes the preparations are placed in distilled water and allowed to remain for one hour or longer. Casein and paracasein are thus stained a pale blue or violet, while similar bodies are practically all colored a light green, or more rarely a yellowish green.

Determination of the Residual Albumin (Koziczkowsky).—The patient is placed upon a test diet very similar to that of Schmidt and Strassburger, consisting of 1½ liters of milk, ¼ liter of bouillon, 6 pieces of Zwieback, 40 grams of oatmeal, 40 grams of butter, 2 eggs, 30 grams of finely hashed meat, and 200 grams of potato. The feces are previously demarcated by giving 0.3 gram of powdered carmine.

Two portions of stool, each representing 2 grams of dried feces,[1] are placed upon nitrogen-free filters and washed successively with ordinary ethyl alcohol (93 to 94 per cent.), absolute alcohol, and 3 per cent. hydrochloric acid. One portion (A) is then mixed with 50 c.c. of a digestive mixture of the following composition:

3 per cent. solution of hydrochloric acid	10.0
Pepsin	30.0
Water	100.0

The second portion (B) is suspended in a corresponding amount of dilute hydrochloric acid without pepsin. The total acidity and amount of free hydrochloric acid are then estimated in each by titrating with $\frac{n}{10}$ alkali solution, after which both specimens are corked and placed in the incubator over night, at 37° C. The next day the total acidity and free acid are again estimated. The difference in the amount of free acid in specimen A indicates the amount which was used in the digestion of the albumins present, and thus serves as an index of their quantity; normally this corresponds to from 15 to 18 c.c. of $\frac{1}{10}$ normal alkali. The difference in the amount of free acid in specimen B is referable to the action of proteolytic ferments (pepsin) in the feces *per se*. Normally this rarely exceeds 2 to 3 c.c. $\frac{1}{10}$ normal solution.

Morphological Elements Derived from the Alimentary Canal.

Epithelium.—Well-preserved cylindrical or goblet cells are only exceptionally found in the feces, while transition forms from the normal cells to mere spindles, in which a nucleus can no longer be recognized, are observed quite constantly. These degenerative changes, according to Nothnagel,[2] are the result of an abstraction of water from the cells, which may alter their appearance to an extent that only the experienced eye is capable of recognizing their true character. Pavement epithelial cells, when present, are derived from the anal orifice.

Epithelial cells when present in large numbers always indicate an inflammatory condition of some portion of the intestinal tract.

Cylindrical epithelial cells are found in abundance in all inflammatory conditions affecting the intestinal mucosa. They are almost exclusively seen embedded in mucus, and it is interesting to note that the cloudy appearance of the mucus is referable to the presence of these elements, and not to leukocytes, as is the case in the sputum.

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[1] 1 gram of formed stool represents 0.3 gram. of the dried substance; 1 gram of semiliquid stool (good fat absorption) equals about 0.25 to 0.27 gram; 1 gram of semiliquid stool (with poor fat absorption) equals about 0.116 gram of dry material.

[2] Beiträge z. Physiol. u. Pathol. d. Darmes, Hirschwald, Berlin, 1884, and Spezielle Pathol. u. Therap., Hölder, Wien, 1895, vol. xvii, pt. i.

When bile-stained specimens are met with, the conclusion is justifiable that the small intestine is involved.

Epithelioid cells may be found in carcinoma of the rectum.

Leukocytes.—Leukocytes are almost always absent in normal stools or present only in very small numbers. Large numbers usually indicate a severe catarrhal, if not an ulcerative, condition of the intestines. Pure pus in large amounts is observed especially in dysentery and in cases in which abscesses have perforated into the gut from adjacent organs or cavities.

Red Blood Corpuscles.—Unaltered red blood corpuscles, according to Nothnagel, are but rarely observed in the feces, no matter how intensely red they may be colored, providing that an ulcerative process affecting the colon or the rectum can be excluded; in that case, as in the severer forms of dysentery, large numbers may be observed. If the hemorrhage has occurred higher up in the intestine, large and small masses of a brownish-red color are seen, which consist of hematoidin. They are mostly amorphous, but in some specimens the characteristic rhombic crystals may be observed. In general, it may be said that the higher the seat of the hemorrhage the darker will be the color of the pigment, and the less the chances of finding well-defined red corpuscles. In such cases recourse must be had to the tests for occult blood (which see).

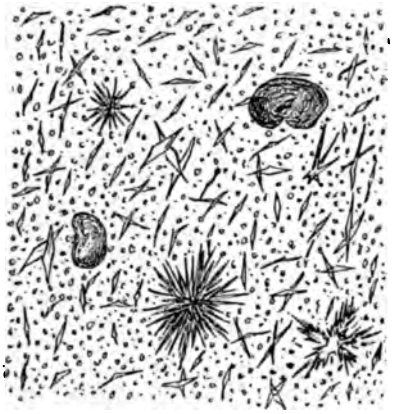

FIG. 74.—Fatty crystals obtained from the feces.

Crystals.—Needle-like crystals of free fatty acids, and the calcium and magnesium salts of the higher members of this group, occurring either singly or arranged in sheaves, may be found in every stool (Fig. 74). They are of no significance unless present in large numbers. Nothnagel speaks of the frequent occurrence of certain calcium salts (of fatty acids, as he believes) in normal as well as pathological stools. He states that they are almost always bile-stained, and occur in irregular, sometimes elliptical, oval, or circular masses, in which a crystalline structure cannot be distinguished. They are apparently of no importance. Quite common, also, are crystals of neutral calcium phosphate and ammoniomagnesium phosphate, the former occurring in the form of more or less well-defined, wedge-shaped crystals collected into rosettes, the latter presenting the well-known coffin-shape when the stool is mushy, while in firm stools irregular fragments mostly are found. At one time the ammonio-magnesium phosphate crystals were supposed to be characteristic of typhoid stools, but it is now known that they occur in normal feces, as well as under the most varied pathological conditions. Their presence is hence of no diagnostic significance. It is important to note that the neutral phosphates are never stained by bile pigment, and the triple phosphates only in rare instances. Both are easily soluble in acetic acid. Crystals of calcium oxalate may be found in abundance following the ingestion of certain vegetables, such as sorrel and spinach. They are usually found embedded in the vegetable debris. They are readily recognized by their characteristic envelope form, their insolubility in acetic acid, and their solubility in hydrochloric acid. Not infrequently they are bile-stained.

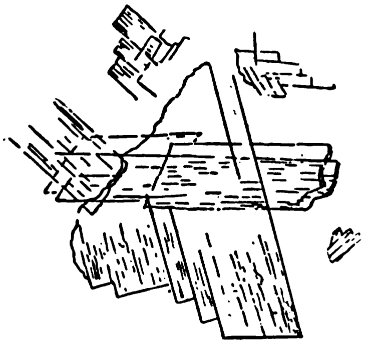

FIG. 75.—Cholesterin crystals.

Calcium lactate is frequently seen in the stools of children receiving a milk diet; it occurs in the form of sheaves composed of radiating needles. Calcium carbonate is rarely observed, but occasionally occurs in the form of amorphous granules or dumb-bell-shaped crystals. Calcium sulphate crystals are likewise rare, but may be produced artificially by the addition of sulphuric acid, when beautiful needles and platelets may be observed. Cholesterin, while always present in solution, is rarely observed in crystalline form (Fig. 75). Hematoidin crystals are never found in normal stools. Charcot-Leyden crystals, according to my experience, are not found in normal stools. They have been described in cases of typhoid fever, dysentery, and phthisis, but are rare in these diseases. In uncinariasis they are more frequently seen, but not in every case. Often they only form after the stool has been kept for some time. They are more likely to be encountered when there are many eggs present than in milder cases. They have further been seen in association with Ascaris lumbricoides, Oxyuris vermicularis, Tænia solium and saginata. In cases of trichocephalus they are but rarely seen, while they are always absent in the case of Tænia nana. According to Leichtenstern[1] their persistence in the feces after the evacuation of what would appear to be a complete tænia should be regarded as indicating the non-removal of the head. In amebic colitis these crystals have also been observed by Lewis, Lafleur, Amberg, myself, and others.

Mucus.—Small hyaline particles of mucus, visible only with the microscope, are not infrequently met with under pathological conditions, and are of diagnostic significance. When bile-stained, their presence is always indicative of disease of the small intestine proper, while colorless particles point to a catarrhal condition of the upper portion of the large intestine or the lower portion of the small intestine. Beginners should be careful not to mistake apparently hyaline particles of vegetable residue for mucus. Mucus never yields a blue color when treated with iodine, or iodine and sulphuric acid, and examination with a higher power will show the entire absence of any definite structure. Both forms, viz., colorless and colored particles, are found intimately mixed with the feces, and may be very abundant. In addition to these forms Nothnagel has described the occasional occurrence of large numbers of roundish or irregular, very pale hyaline or opaque formations, which are devoid of all structure. Some specimens are homogeneous, while others present a distinct rimous appearance. They have been found only in liquid stools, and are apparently of no diagnostic significance. To judge from their optic behavior, they probably consist of mucus.[2]

BACTERIOLOGY OF THE FECES.

The bacteria are the microörganisms κατ' ἐξοχήν which are found in the feces. Their number is truly enormous. Sucksdorff found in his own person that on an average 53,124,000,000 were eliminated in the twenty-four hours under normal conditions. If we recall the strongly bactericidal power of the gastric juice, such an observation must at first sight appear surprising. It should be remembered, however, that large amounts of the ingesta are carried into the small intestine at a time when hydrochloric acid has not yet appeared in the free state.

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[1] Deutsch. med. Woch., 1885, vol. xi, Nos. 29 and 30; ibid., 1886, vol. xii, Nos. 11 to 14; ibid., 1887, pp. 565, 594, 620, 645, 669, 691, and 712.

[2] A. Schmidt, "Ueber Schleim im Stuhlgang," Zeit. f. klin. Med., vol. xxxii, p. 260.

On the whole, the bacteriological flora of the intestinal contents is fairly constant, but, as in the other cavities and channels of the body where bacteria are invariably met with, transient guests are also not uncommon. The majority of the bacteria which are here encountered are, as a general rule, harmless; but it is important to note that under suitable conditions a number of these may develop pathogenic properties. Broadly speaking, the bacteria which may be found in the feces can be divided into two classes, viz., into alkali producers and acid producers. Many of these forms have been described for the first time by Ford,[1] and the following schema, which gives a very good idea of the numerous individual types, although not complete, is taken from his excellent work:

Alkali Producers.

GROUP I. Organisms producing alkali in litmus milk; not liquefying any media; not fermenting carbohydrates to the point of acidity. *Fecalis alkaligenes*, or *Petruschky group*. Represented by:

Bacillus alkaligenes.

GROUP II. Organisms producing alkali; not liquefying any media; fermenting carbohydrates to the point of acidity, but no gas. *Dysentericus*, or *Shiga group*. Represented by:

Bacillus dysenteriæ.
Bacillus pseudodysentericus, Müller.
Bacillus typhi.
Bacillus acidophilus.

GROUP III. Organisms producing alkali; not liquefying any media; fermenting the carbohydrates with the production of acidity and gas. *Hog cholera*, or *suipestifer group*. Represented by:

Bacillus alkalescens, Ford; ferments dextrose, saccharose, and lactose.
Bacillus subalkalescens, Ford; ferments dextrose, saccharose, and lactose.
Bacillus enteritidis, Gärtner; ferments dextrose.
Bacillus galactophilus, Ford; ferments saccharose and lactose.

GROUP IV. Organisms producing alkali; liquefying gelatin; fermenting carbohydrates with the production of acid and gas. *Entericus group*. Represented by:

Bacillus entericus, Ford; ferments dextrose, saccharose, and lactose.
Bacillus subentericus, Ford; ferments dextrose and lactose.

GROUP V. Organisms producing alkali; liquefying gelatin, casein, and blood serum; fermenting carbohydrates with the production of acid and gas. *Proteus*, or *Hauser group*. Represented by:

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[1] Studies from the Royal Victoria Hospital, Montreal, vol. i, No. 5, and from the Rockefeller Institute for Medical Research, vol. ii.

Bacillus plebeius, Ford; ferments dextrose, saccharose, and lactose.

Bacillus infrequens, Ford; ferments dextrose and lactose.

Bacillus vulgaris, Hauser; ferments dextrose and saccharose.

GROUP VI. Organisms producing alkali; liquefying various media, but not fermenting carbohydrates to the point of acidity. *Booker group.* Represented by:

Bacillus recti, Ford; liquefies gelatin.

Bacillus pylori, Ford; liquefies gelatin and casein.

Bacillus ceci, Ford; liquefies gelatin, casein, and blood serum.

Bacillus Bookeri, Ford; liquefies gelatin, casein, and blood serum.

Bacillus pyocyaneus.

Acid Producers.

GROUP I. Organisms acidifying and coagulating milk; not liquefying any media; not fermenting carbohydrates to the point of acidity. *Fecalis oxygenes,* or *Bienstock group.* Represented by:

Bacterium oxygenes, Ford.

Bacterium Bienstock, Schröter.

GROUP II. Organisms acidifying and coagulating milk; not liquefying any media; fermenting carbohydrates to the point of acidity, but no gas. *Acidoformans,* or *Sternberg group.* Represented by:

Bacillus oxyphilus, Ford.

Bacterium acidoformans, Sternberg.

Bacterium minutissimum, Migula.

GROUP III. Organisms acidifying and coagulating milk; not liquefying any media; fermenting carbohydrates with the production of acidity and gas. *Coli,* or *Escherich group.* Represented by:

Bacillus coli, Migula; ferments dextrose and lactose.

Bacillus communior, Ford; ferments dextrose, saccharose, and lactose.

Bacterium aërogenes, Migula; ferments dextrose, saccharose, and lactose.

Bacterium duodenale, Ford; ferments dextrose and lactose.

GROUP IV. Organisms acidifying and coagulating milk; liquefying gelatin and fermenting the carbohydrates with the production of acidity and gas. *Liquefaciens,* or *Eisenberg group.* Represented by:

Bacillus gastricus, Ford; ferments dextrose, saccharose, and lactose.

Bacillus subgastricus, Ford; ferments dextrose and lactose.

Bacterium liquefaciens, Eisenberg; ferments dextrose, saccharose, and lactose.

Bacterium subliquefaciens, Ford; ferments dextrose and lactose.

GROUP V. Organisms acidifying and coagulating milk; liquefying gelatin, casein, and blood serum, and fermenting the carbohydrates with the production of acidity and gas. *Cloacæ,* or *Jordan group.* Represented by:

Bacillus cloacæ, Jordan; ferments dextrose, saccharose, and lactose.
Bacillus subcloacæ, Ford; ferments dextrose and lactose.
Bacillus iliacus, Ford; ferments dextrose and saccharose.

GROUP VI. Organisms acidifying and coagulating milk; liquefying various media; fermenting the carbohydrates with the production of acidity, but no gas. *Dubius,* or *Kruse group.* Represented by:

Bacillus chylogenes, Ford; liquefies gelatin.
Bacterium chymogenes, Ford; liquefies gelatin.
Bacillus leporis, Migula; liquefies gelatin and blood serum.
Bacillus dubius, Kruse; liquefies gelatin, blood serum, and casein.
Bacillus jejunalis; liquefies gelatin, blood serum, and casein.

All the above are non-pigment, non-spore bearing organisms. In addition to these the following pigment-producing and spore-bearing organisms have been isolated:

Pseudomonas aëruginosa, Schröter.
Pseudomonas ovalis, Ravenel.
Bacterium Havaniense, Sternberg.
Bacterium lutescens, Migula.
Bacterium anthracoides, Hüppe and Wood.
Bacterium implectans, Burchard.
Bacillus cereus, Frankland.
Bacillus mycoides, Flügge.

The above list indicates the various organisms which have thus far been isolated from the intestinal contents. Many other forms exist, but have not yet been cultivated, as they do not grow on the artificial media which are now in use.

The more important members of the series are described below.

Fungi.—Fungi, with the exception, perhaps, of the Oidium albicans, which has at times been observed, are rarely found in the feces.

Schizomycetes.—Saccharomyces cerevisiæ is one of the normal constituents of the feces, and is found in its characteristic forms, three or four buds, however, being but ordinarily observed. Owing to the glycogen present in their substance, they assume a mahogany color when treated with a solution of iodopotassic iodide. They should not be confounded with a class of bacteria which closely resemble the saccharomyces in general appearance, but are colored blue when treated in the same manner.

Bacillus dysenteriæ, Shiga.—This organism is now generally regarded as the specific cause of the common form of acute dysentery which prevails not only in the tropics, but also in the United States and Europe. It was discovered by Shiga in Japan in 1897, and is identical with the organism obtained by Flexner and Strong in the Philippines and Porto Rico, by Vedder and Duval in the United States, and by Kruse in Germany. From the researches of Bassett and Duval it further appears that the same bacillus is also responsible for the common form of infantile summer diarrhea which prevails in warm countries.

In the United States the Flexner-Harris type is by far the most common in infantile cases. In the collection of 237 cases reported by Holt this type was found in 207, while the true Shiga bacillus was present in only 23; both organisms were found in 7 cases.

The bacillus in question is a short rod with rounded ends, and resembles the typhoid bacillus and most members of the colon group. It is probably non-motile so far as active locomotion is concerned, but it is possessed of a high degree of molecular motion. It stains with the usual basic dyes and is decolorized by Gram's method.

Upon gelatin plates at room temperature there appear, after a few days, small round dots, which, magnified under low powers, are slightly yellow and finely granular. After a few days they increase in size; the middle portion of the colonies then appears darker under a low power, while the outer zone appears brighter and more seed-like. The superficial and deeper colonies show no marked variation. In stab cultures on gelatin a whitish strand forms the whole length of the stab. The gelatin is not liquefied.

After twenty-four hours in the incubator single colonies upon slanted agar appear moist, bluish, and partially translucent. After two days they present a combination of a middle dark and a peripheral bright, sharply defined zone.

The growth on glycerin agar is slightly more abundant than on ordinary agar. The organism grows on blood serum without liquefying it.

In the stab cultures on glucose agar there is formed along the whole line of the puncture a thick, gray-white strand without the development of gas. Upon potato after twenty-four hours in the incubator there is hardly any perceptible growth, only the surface appears slightly shiny. After two days this changes to a yellow brown. In the course of a week the growth is heavier and of a deeper brown color. Bouillon cultures show after a day in the incubator a somewhat intense cloudiness, with a moderate precipitate. No pellicle is formed on the surface. No indol reaction is present. Litmus milk after twenty-four hours appears reddish; otherwise, however, it undergoes no change. The milk never coagulates.

The bacillus is pathogenic for mice, rabbits, and guinea-pigs. It is agglutinated by the patient's blood serum, and it is interesting to note that this reaction is obtained only with cases definitely known to have been infected with the microörganism in question.

Isolation of Shiga's Bacillus from the Feces.—The fecal matter is collected on a sterile pad, or, still better, obtained from the rectum by curettage. A bouillon culture is prepared and from this agar tubes are inoculated *as soon as possible.* The agar should be just acid to phenolphthalein (slightly alkaline to litmus), and is plated at once. Ten plates, variously diluted, are conveniently used. After twenty-four hours in the incubator at 37° to 38° C. all colonies are marked on the plates which have developed by that time. The plates are returned to the incubator. After further twenty-four hours tubes of glucose agar and litmus-mannite agar are inoculated from those colonies which have grown in the second twenty-four hours—*i. e.*, those colonies which have not been marked. At the end of another twenty-four hours in the incubator all those tubes are rejected in which fermentation has taken place. From those tubes in which this has not occurred, litmus milk, litmus mannite, and bouillon are inoculated. The Shiga bacillus will at first render the milk slightly acid, but later it becomes alkaline. Litmus mannite remains unchanged with the Shiga strain, while the Flexner-Harris type (the American acid type) turns it red. Ultimate identification is made by the agglutination test in various dilutions (1 to 50 to 1 to 100) reading the results after two hours.[1]

LITERATURE.—K. Shiga, Centralbl. f. Bakt., Parasit. u. Infectionskrankh., 1898, vol. xxiv. R. P. Strong and Musgrave, "Preliminary Note regarding the Ætiology of the Dysenteries of Manila," Report of the Surgeon-general of the Army, Washington, 1900, p. 251. S. Flexner, "On the Etiology of Tropical Dysentery," Bull. Johns Hopkins Hosp., 1900, p. 231. Vedder and Duval, "The Etiology of Acute Dysentery in the United States," Jour. Exper. Med., vol. vi, p. 181. Duval and Bassett, Amer. Med., 1902, iv, p. 417 (preliminary report).

Bacillus typhi, Eberth.—The typhoid bacillus can only be demonstrated in the feces by cultural method which will enable its separation from other members of the colon group. To this end many different methods have been suggested.

Combined Malachite-green Method of Lentz and the Method of v. Drigalski and Conradi.—This method is probably the most useful and extensively employed abroad. The media are prepared as described elsewhere (see Media). Two plates of Drigalski's medium are prepared in large Petri dishes, using 20 to 25 c.c. of the medium for each plate (15 to 20 cm. diameter); these are left uncovered until the steam has evaporated and the agar is quite firm. Contamination by the organisms of the air does not occur owing to the presence of the cresyl violet in the medium. The malachite-green medium is already plated when made (see Media). The stool is stirred up well with a small amount of sterile normal salt solution. Of this material about 0.5 c.c. is placed on the green plate and smeared over its surface with a glass rod, which is conveniently bent at an angle about one inch from the end. Without sterilizing, the same rod is then smeared over the first Drigalski plate and hence over the second. After this all three are allowed to become perfectly dry by standing open in the air, when they are incubated for twenty to twenty-four hours. Plates 2 and 3 are now examined with a hand lens, placing them, if possible, in such a position that light reflected from a wall falls upon them. The colon colonies are more or less red in color, not transparent, and measure 1 to 3 mm. in diameter. The typhoid colonies are bluish with a violet shade and resemble drops of dew. If such are found they are further identified as follows: A tiny bit of the colony is placed on a slide and mixed with a drop of a highly active hundredfold dilution of typhoid (viz., paratyphoid) serum. Agglutination may be observed with a hand lens or a low power of the microscope. If this occurs further tests are made by inoculating ordinary agar, litmus whey, and neutral red agar (see Culture Media).

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[1] For a detailed account of the different varieties of the dysentery bacillus and dysentery-like organisms see J. C. Torrey, Journ. Exper. Med., 1905, vol vii, p. 365.

If no colonies are found on the Drigalski medium which resemble typhoid bacilli, the green plate is flooded with sterile normal salt solution, gently agitated, and set aside for a few minutes. In this manner the typhoid and paratyphoid colonies, which are more delicate than the colon colonies, come to be disseminated in the fluid, while the latter sink to the bottom. With the glass spatula two more Drigalski plates are then prepared from the salt solution, incubated for twenty to twenty-four hours, and examined as described.

If urine is to be examined in the place of feces, several drops are placed on the green plate and one drop only on the Drigalski plate. The procedure otherwise is the same.

Blood is examined in a similar way, but must first be diluted in sterile bouillon to eliminate the bactericidal substances that are present (5 to 200).

In pure cultures the typhoid bacilli present the following features: They occur in the form of rods of almost one-third the size of a red blood corpuscle, or in threads composed of several rods joined end to end (Figs. 76 and 77). Their ends are rounded; their length is equivalent to about three times their breadth. They are actively motile and provided with polar as well as lateral flagella. They grow very readily on bouillon-peptone gelatin, and after twenty-four hours colonies begin to appear. When slightly magnified, these present a faintly yellowish color; macroscopically they are barely visible. The organism does not form spores, but when kept at a temperature of 37° C., and especially when grown on media colored with phloxin red or benzopurpurin, polar bodies are observed which were formerly mistaken for spores. Gelatin is not liquefied; the growth is white and delicate, both along the line of the stab and on the surface. Cultivation in glucose bouillon, or glucose agar, does not give rise to the formation of gas, but after twenty-four hours the entire fluid becomes turbid. Milk is rendered feebly acid, but is not coagulated. No indol reaction is obtained when the organism is grown on peptone-containing media. On potato a very faint, whitish, almost invisible growth takes place. When grown on gelatin or agar that has been colored with neutral red, the typhoid bacillus causes no change in color. Absolute identification is possible by means of Pfeiffer's agglutination test (see Widal's reaction).

In cases of *paratyphoid* infection the corresponding organism may be found in the feces (see Blood).

Bacillus acidophilus, Moro.[1]—This organism has been described by Moro as occurring in the stools of breast-fed infants, in which it normally predominates over all other forms; under pathological conditions, on the other hand, as also in the stools of children which have been fed with cows' milk, their number is found diminished, while the members of the coli group enter into the foreground. Beyond the stools the bacillus has been found in the outer portion of the secretory duct of the human mammary gland, in the milk, and the skin of the nipple and its immediate surroundings. It is apparently not pathogenic.

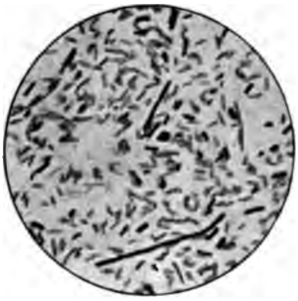

FIG. 76.—Typhoid bacilli from nutrient agar. × 1100 diameters. (Park.)

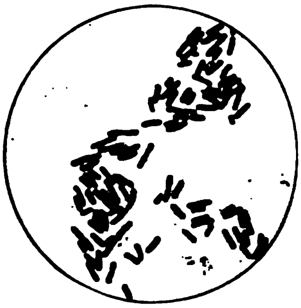

FIG. 77.—Typhoid bacilli from nutrient gelatin. × 1100 diameters. (Park.)

The organism occurs in the form of slight rods measuring 1.5 μ to 2 μ in length, by 0.6 μ to 0.9 μ in breadth. It is non-motile. It is not decolorized by Gram's method, but loses this property after from thirty-six hours to nine days. The best growths are obtained on beer-wort bouillon and common bouillon when acidified with a mineral acid; the acidity of 10 c.c. of the medium may correspond to 10 c.c. of a decinormal solution of potassium hydrate. The optimum temperature is 37° C.; between 20° C. and 22° C. no growth occurs. On the various agar slants imperfect development takes place; on potato the organism does not grow. It is an active acid producer, but does not give rise to the formation of gas; with Escherich's stain it is colored blue.

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[1] "Ein Beitrag zur Kenntniss der normalen Darmbacterien des Säuglings," Jahrbuch f. Kinderheilk., vol. lii. Also: "Ueber die nach Gram färbbaren Bacillen d. Säuglingstuhles." Wien. klin. Woch., 1900, No. 5.

Escherich's Stain.—This stain is now extensively used by pediatrists in order to ascertain any deviations from the normal in the flora of the feces. Under strictly normal conditions the bacilli which are found in the stools of breast-fed children are thus nearly all colored blue (these are essentially the anaërobic Bacillus bifidus communis, and the aërobic Bacillus acidophilus), while red bacilli (Bacillus coli communis and Bacillus lactis aërogenes) are but little numerous. In the case of infants, on the other hand, which are fed exculsively on cows' milk, the red bacilli predominate, while in mixed feeding the blue enter into the foreground in about the proportion in which breast milk is employed. The red bacilli belong to the coli group. These further predominate, or may be found exclusively, if for any reason intestinal digestion is impaired. Staphylococci, streptococci, etc., when simultaneously present, are in either event stained blue. In staphylococcus enteritis the blue bacilli which normally exist in the stools of breast-fed infants are almost entirely replaced by staphylococci. At the beginning of the enteritis they are not numerous, but they increase during the progress of the disease, and finally disappear when the child recovers.

In staining, the following solutions are employed:

1. An aqueous solution of gentian violet (5 to 200). This is boiled for one-half hour and is then filtered; it keeps for a long time.
2. A mixture containing 11 parts of absolute alcohol and 3 parts of oil of anilin.

1 and 2 are mixed in the proportion of 8.5 to 1.5; the resulting solution keeps for from two to three weeks, but not longer.

3. A solution of iodopotassic iodide containing 1 part of iodine and 2 parts of potassium iodide in 60 parts of water.
4. A mixture of equal parts of oil of aniline and xylol.
5. A concentrated alcoholic solution of fuchsin, diluted with an equal volume of absolute alcohol.

A bit of the stool is spread upon a slide in as thin a layer as possible. After drying in the air the specimen is fixed by passing through the flame of a Bunsen burner. It is then stained for a few seconds with the mixture of 1 and 2, blotted, placed in the iodine solution for a few seconds, blotted again, decolorized with 4 until a notable extraction of color no longer occurs. It is washed with xylol, dried, and finally stained for a few seconds with the fuchsin solution, washed with water, blotted, and is then ready for examination.

Bacillus (Proteus) vulgaris, Hauser.—This organism, while usually regarded as non-pathogenic, should be numbered among the bacteria which may at times develop pathogenic properties. Baginsky and Booker have frequently found it in the stools in cases of infantile summer diarrhea. Escherich observed it at times in the meconium. Brudzinski examined the dyspeptic and fetid stools of a number of artificially fed infants in Escherich's clinic, and in all the cases found the proteus. Others have encountered it in inflammatory conditions of exposed surfaces, in appendicitis, in perforative peritonitis, and even in closed abscesses, either alone or in association with other bacteria (Welch). A mixed infection of the proteus with Löffler's bacillus has also been observed. The organism forms rods, measuring about 0.25 μ in diameter, while their length is variable; at times a more roundish form is observed; at others rods measuring from 1.25 μ to 3.75 μ in length, or even long threads. They are readily stained, but are easily decolorized by alcohol or Gram's method. Most characteristic is their growth upon nutrient gelatin. At the temperature of the room little depressions will be observed after six to eight hours, which are surrounded by a narrow zone of bacilli from which a thin, wide film, provided with irregular projections, extends over the culture medium. From this film islets become separated, which slowly extend over the gelatin and cause its liquefaction. The organism is motile. It decomposes urea and causes albuminous putrefaction. The nitroso-indol reaction is readily obtained in bouillon cultures. In boiled milk the organism grows well, while in fresh milk it develops only irregularly, and in acid milk no growth takes place at all.

Bacillus pyocyaneus.—The Bacillus pyocyaneus has repeatedly been isolated from the stools of dysenteric patients, and has been proved the cause of several epidemics. The organism in question is a small motile bacillus measuring from 1 μ to 2 μ in length by 0.3 μ to 0.5 μ in breadth. It sometimes occurs in short chains, but is usually single. It is stained with the common aniline dyes, and is decolorized with Gram's method. It grows on the usual culture media, and liquefies gelatin. In 2 per cent. glucose bouillon no fermentation takes place. Litmus milk is curdled in about forty-eight hours. Some varieties produce indol. Most characteristic is the production of certain pigments, viz., pyocyanin and a fluorescent, bluish-green pigment which is common to almost all varieties.[1]

The **Bacillus coli communis**,[2] while constantly present in normal feces, is described at this place, as modern investigations have shown that it may at times develop pathogenic properties. It has been found in the pus in cases of purulent perforating peritonitis, angiocholitis, pyelonephritis, etc.; it is frequently found infecting the bladder and the pelvis of the kidney, and, as indicated elsewhere, at times forms the nucleus of gallstones. It occurs in the form of delicate or coarse rods, measuring about 0.4 μ in length, which manifest a certain degree of motility, due to the presence of one or two polar flagella. The organism is stained by the usual aniline dyes, and is decolorized by Gram's method. The colonies upon gelatin closely resemble those of the bacillus of typhoid fever, forming small whitish specks *in* the gelatin, and delicate films with serrated borders *upon* the same medium, which, moreover, is not liquefied. On potato the organism forms a brownish pellicle, while the growth of the typhoid bacillus is nearly transparent. As in the case of the cholera bacillus, the nitroso-indol reaction can be obtained when the organism is grown upon peptone-containing media.[1] In solutions of glucose active fermentation takes place. Litmus milk is rendered acid and is coagulated. Important also is the behavior of the organism when grown on gelatin or agar that has been colored with neutral red; in contradistinction to the typhoid bacillus, the colon bacillus then causes an intense green fluorescence.

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[1] A. J. Lartigau, "A Contribution to the Study of the Pathogenesis of the Bacillus Pyocyaneus," etc., Jour. Exper. Med., 1898, No. 6.

[2] Flügge, Die Microörganismen.

The **Bacillus lactis aërogenes** (Escherich) closely resembles the organism just described, and may also at times develop pathogenic properties. It is seen quite constantly in the stools of sucklings, but may also be met with in those of adults. It occurs in the form of rather stout rods, which frequently lie in pairs, resembling diplococci. The organism is non-motile. Like the Bacillus coli communis, it is decolorized by Gram's method. In plate cultures it forms a dense white film; in stab cultures a chain of white colonies resembling beads is seen. In the latter, moreover, if the stab is closed, bubbles of gas will be seen to form, which rapidly increase in number and size. Milk is coagulated in large lumps in twenty-four hours; at the same time the formation of gas is much more intense than in the case of the Bacillus coli communis.

The Comma Bacillus.—The first detailed studies of the organisms found in cholera stools were made in 1883 by the members of the French and German commissions sent to Egypt to investigate the nature of the dreaded disease. The results of their work were first published by Koch in his report to the Berlin Sanitary Office in 1883, and in 1884 by Strauss, Roux, Nocard, and Thuillier.

The clinical recognition of cholera Asiatica has now become a simple matter since Pfeiffer has demonstrated that the blood serum of cholera patients possesses the property of causing arrest of motility and agglutination of the specific bacilli. Ordinary bouillon cultures, however, can usually not be employed, as particles of the film when broken up may easily be mistaken for agglutinated bacilli. It is best in every case to make use of agar cultures sixteen to twenty-four hours old, and to prepare emulsions in bouillon or normal salt solution as occasion requires. The emulsion, moreover, should always be examined microscopically before use, so as to ensure the absence of any conglomeration of bacilli. The blood is then diluted in the proportion of 1 to 10 or 1 to 15. If the test-tube method is employed, the tubes should be kept in the incubator (37° C.) for only one or two hours. Upon the slide the reaction is obtained in from five to twenty minutes. If no distinct agglutination is observed at the end of one hour, the diagnosis of cholera is rendered improbable. Dried blood retains its agglutinating properties for a considerable length of time, and may also be used for examination.

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[1] The test for indol is very conveniently made by adding a few drops of Ehrlich's dimethyl-amino-benzaldehyde solution (see Urine) to a culture of the organism in Dunham's solution which has grown for four or five days. On shaking, and especially on heating, a cherry-red color develops.

The comma bacillus is a slightly arched or half-moon-shaped little rod, and is somewhat shorter than the tubercle bacillus (Fig. 78). Occasionally two are placed end to end with their convexities in opposite directions, thus presenting the appearance of the letter S. They are provided with flagella. Koch detected these bacilli in the intestinal contents and feces, but rarely in the vomited matter, in Asiatic cholera only. In the stools they at times occur in such numbers as to constitute pure cultures. In plate cultures kept at a temperature of 22° C. white colonies with serrated borders may be observed after twenty-four hours. The color of such a colony is slightly yellow or rose red, its central portion gradually assuming a deeper tint, and finally becoming liquefied. Upon agar plates the bacilli form a grayish-yellow, irregular, slimy coating, but do not liquefy the culture medium. In stab cultures, after twenty-four hours, a whitish color may be observed along the line of the stab; around this there is found a funnel-shaped depression, which gradually increases in size and apparently contains a bubble of gas. The upper portion of the culture medium at the same time becomes liquefied while the lower portion remains solid for days. In a suspended drop spirochete-like spirals are observed at the margins, which often present as many as twenty distinct arches[1]

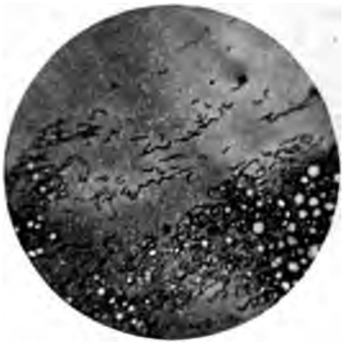

FIG. 78.—Cholera spirilla preparation from gelatin-plate culture of cholera. × 800 diameters. (Park.)

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[1] R. Koch, Berlin. klin. Woch., 1884, vol. xxi, pp. 477, 493, 509.

Closely related to Koch's comma bacillus is the *bacillus of Finkler and Prior*,[1] discovered in 1884 and 1885. It is distinguished from the former by the following characteristics: it is larger and thicker than the comma bacillus; the colonies on gelatin plate cultures show equally round and sharp-edged forms, which present a granular appearance under a low or medium power, and are usually of a brown color. The organism liquefies gelatin very rapidly, a penetrating, excessively fetid odor being developed at the same time. In stab cultures the bacillus of cholera Asiatica forms a funnel-shaped depression, while the bacillus of Finkler and Prior forms a stocking-like depression.

Tubercle bacilli, when present in the feces, are indicative of intestinal tuberculosis, providing they are observed upon repeated examination and there are clinical symptoms pointing to the bowels as the seat of the disease; otherwise they may be referable to swallowed sputa. They may be demonstrated as described in the chapter on Sputum.

ANIMAL PARASITOLOGY OF THE FECES.

The animal parasites which may be met with in the feces are classified as follows:

I.—Protozoa:
    1. Rhizopoda,
        Monera,
        Amœbina: Amœba coli.
    2. Sporozoa, S. gregarina,
        Coccidia,
    3. Infusoria,
        a. Ciliata,
            Holotricha: Balantidium coli.
        b. Flagellata.
            Monadina,
                Cercomonadina: Cercomonas.
            Isomastigoda.
                Tetramitina: Trichomonas.
            Polymastigina: Megastoma.
II.—Vermes:
    Platodes,
        Cestodes,
            Tænia saginata.
                Tænia solium.
                Tænia nana.
                Tænia diminuta.
                Tænia cucumerina.
                Bothriocephalus latus.
                Krabbea grandis.

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[1] Finkler, Deutsch. med. Woch., Tageblatt der Naturforscherversammlung, 1884, vol. x, p. 36, and 1885, p. 438. Finkler u. Prior, Ergänzungsheft z. Centralbl. f. allg. Gesundheitspflege, 1885, vol. i.

- **Trematodes,**
    - **Distoma hepaticum.**
    - **Distoma lanceolatum.**
    - **Distoma Buskii.**
    - **Distoma sibiricum.**
    - **Distoma spatulatum.**
    - **Distoma conjunctum.**
    - **Distoma heterophyes.**
    - **Amphistoma hominis.**
    - **Distoma hæmatobium.**
    - **Distoma pulmonale.**
- Annelides,
    - Nematodes,
        - " **Ascarides,**
            - **Ascaris lumbricoides.**
            - **Ascaris mystax.**
            - **Ascaris maritima.**
            - **Oxyuris vermicularis.**
        - **Strongyloides,**
            - **Ankylostomum duodenale.**
        - Trichotrachelides,
            - **Trichocephalus hominis.**
            - Trichina **spiralis.**
        - **Rhabdonema strongyloides,**
            - **Anguillula intestinalis.**

Protozoa.—The *rhizopoda* are essentially characterized by the fact that locomotion does not take place by the aid of independent organs, but by means of pseudopodia, viz., protoplasmic processes which the animal is capable of protruding from any portion of its body. Six orders have been described by zoölogists, but only one, or possibly two, have thus far been found in the feces.

Whether or not representatives of the *monera* occur in the feces of man is still an open question. If so, they are apparently of no pathological significance.[1]

Of the *amœbina*, on the other hand, a most important member has been found, viz., the Entamœba dysenteriæ.

Entamœba Dysenteriæ, S. Histolytica (Schaudinn): *syn.*, **Amœba Coli** (Lösch).—In 1875 Lösch[2] discovered in the stools of dysenteric patients actively moving cell-like bodies of a roundish, pear-shaped, oval, or irregular form. He did not regard these as the cause of the disease, however, but looked upon them as only accidentally present. Similar bodies were observed in Hong-Kong by Normand in cases of colitis; and also by v. Jaksch. Sansino found them in a case in Cairo, and Koch in East Indian dysentery. It is interesting to note that Koch was the first to suspect the existence of a definite relation between dysentery and these organisms. Cunningham claims to have found amebas frequently in the stools of cholera patients at Calcutta, and Grassi in normal stools, but especially abundant in cases of chronic diarrhea. Whether all these observations are correct, and whether the organisms observed were identical in all cases, is, of course, difficult to say. So much is certain, that the subject was still in a very unsettled state when Kartulis[1] announced "that dysentery and tropical liver abscess associated with dysentery are caused by the presence of the Amœba coli," basing his conclusion upon an examination of 500 cases. The fact that this parasite was absent in all other intestinal diseases, such as typhoid fever, intestinal tuberculosis, the ordinary forms of diarrhea, etc., speaks strongly in favor of Kartulis' view.

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[1] Nothnagel, loc. cit., p. 110. Grassi, cited by Bizzozero. v. **Jaksch, Wien. klin.** Woch., 1888, vol. i, p. 511.

[2] "Massenhafte Entwickelung v. Amöben im Dickdarm," **Virchow's Archiv,** vol. lvi.

In perfect accord with these observations are those made at the Johns Hopkins Hospital.[2] Osler[3] was the first in this country to demonstrate the presence of the Amœba coli in a case of liver abscess, both in the pus of the abscess and in the stools. Stengel, Musser, Dock, and others confirmed these observations, and the pathogenic character of the Amœba coli may now be regarded as an established fact.[4] This statement is based not only upon the few facts, more historical in character than otherwise, which have just been detailed, but rather upon the *ensemble* of collected data, among which the absence of microörganisms other than the ameba in the pus of the liver abscesses, and the constant presence of the latter in such cases, rank among the most important. It is to be noted, however, that different forms of tropical dysentery exist, and that the Amœba coli is essentially associated with the more chronic form, while the acute types are of bacillary origin (see Shiga's bacillus).

The size of the amebas averages 35 μ. When at rest their outline is, as a rule, circular, occasionally ovoid; but when in motion they present the extremely irregular contour of moving ameboid bodies (Plate XIV). The protoplasm can be differentiated into a translucent, homogeneous ectosarc or mobile portion, and a granular endosarc, containing the nucleus, vacuoles, and granules. Within the endosarc the vacuoles constitute the most striking feature. Sometimes the interior seems to be made up of a series of closely set, clear vesicles of pretty uniform size. As a rule, one or two larger vacuoles are present, the edges of which are not infrequently surrounded by fine, dark granules. True contractile vesicles displaying rhythmic pulsations have not been observed, although the vacuoles may at times be seen to undergo changes in size. In some the nucleus is quite distinct, while in others it may be altogether invisible. The protoplasm of the amebas is strongly basophilic.

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[1] "Zur Aetiologie d. Dysenterie in Egypten," etc., Virchow's Archiv, 1885, vol. cv, and 1889, vol. cxviii. Centralbl. f. Bakt. u. Parasit., 1890, vol. vii.

[2] Councilman and Lafleur, "Amœbic Dysentery," Johns Hopkins Hosp. Rep., 1891, vol. ii. C. E. Simon, Johns Hopkins Hosp. Bull., 1890.

[3] Johns Hopkins Hosp. Bull., 1890.

[4] For the more recent literature see especially H. F. Harris, "Amœbic Dysentery," Amer. Jour. Med. Sci., 1898, p. 384.

Most distinctive are the movements of these bodies. From any part of the surface a rounded, hemispherical knob will project, and with a rapid movement the process extends and the granules in the interior flow toward it. In these movements the clear ectosarc seems to play the most important part. The organisms are actively phagocytic and often contain red corpuscles, bacteria, and crystals. Reproduction occurs by fission.

Various attempts have been made to cultivate the Amœba coli, but on the whole the results have not been satisfactory. In every attempt in this direction adequate bacterial symbiosis must be secured. The most comprehensive work in this direction has been done by Musgrave and Clegg. The medium which they recommend has the following composition and is prepared as ordinary agar:

Agar	20.0	pro liter.
Sodium chloride	0.3–0.5	
Beef extract	0.3–0.5	

The final product is most universally satisfactory when 1 per cent. alkaline to phenolphthalein, to which end it is recommended to start with an initial alkalinity of 1.5 per cent.

Tubes of this medium are plated and the surface lightly smeared with material selected from feces containing amebas. The first plates must be watched frequently under the microscope, and as soon as it is found that amebas have developed (twenty-four hours to four or five days) transplants must be made, as otherwise they are liable to die. For further details to this end the reader is referred to Musgrave and Clegg's monograph.[1]

To demonstrate amebas in stools it has been generally suggested to procure bits of mucus or mucopus for examination. Musgrave and Clegg recommend that the patient be given a saline cathartic and that the examination be made from the fluid portion of the stool. Drops of this are mounted, covered with cover-glasses, and examined with a $\frac{1}{6}$. The diagnosis of amebiasis should then only be made if motile amebas are encountered. Resting or encysted forms may be mistaken for epithelial cells, swollen leukocytes, etc.

Not infrequently some of the organisms are found containing one or more red cells. (Plate XIV.)

Staining is not at all essential for the purpose of demonstrating amebas in the stool. The examination of the fresh material is much more satisfactory and far less likely to lead to errors of diagnosis.

Very pretty pictures are obtained by vital staining with neutral red. (Plate XIV.) To this end it is only necessary to run a drop of a dilute solution of the dye under the cover-glass, when it will be seen that the young, actively motile amebas take up the stain without

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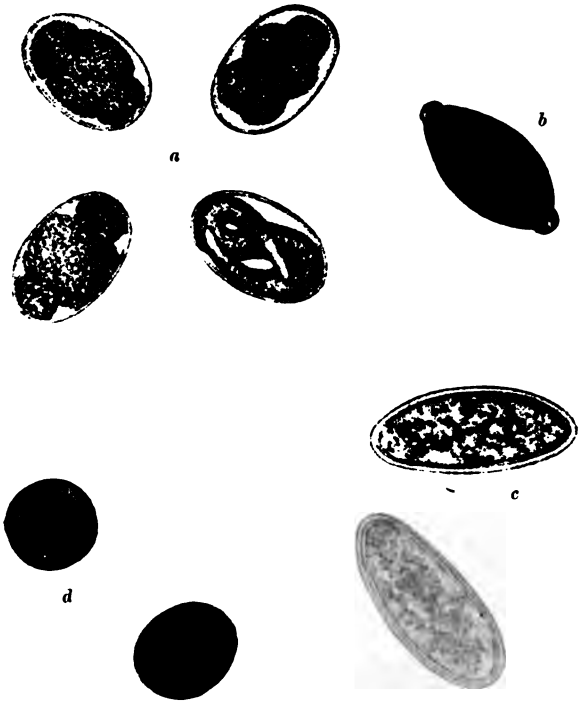
[1] Amebas, Bureau of Government Laboratories. Biological Laboratory of Manila, 1904.

PLATE XIV.

Amœbæ Fed with Neutral Red and Containing Phagocytes and Red Cells.

PLATE XV.



Eggs of Parasites.

Uncinaria americana ; *b*, Trichocephalus dispar ; *c*, Oxyuris vermicularis ; *d*, Tænia saginata.

losing their motility. They can then be readily watched in their movements.

The preparation of stained permanent preparations is not very satisfactory. They are prepared like blood films and colored with one of the modifications of the Romanowsky dye.

When older material only is available it may be difficult to arrive at a satisfactory conclusion. Sometimes it is possible to cause the amebas to move again by warming the stool in an open dish at body temperature, but more often they are dead. Attention should then be especially directed to ameba-like structures containing red blood cells. If such are found the inference that the cell is a dead ameba is usually warrantable.

Entamœba coli (Schaudinn).—This is not to be confused with the Entamœba dysenteriæ. It is smaller than the Entamœba dysenteriæ, the size varying between 10 and 15 μ. It is opaque, gray in color, and provided with a distinct nucleus. The ectoplasm is usually not visible. The movements are much more sluggish and the tendency to phagocytosis much less marked. It is considered to be non-pathogenic. In the Philippines it is apparently quite common. Craig[1] finds 65 per cent. of normal individuals infected with it, but uses saline purgatives to produce diarrheal discharges, as recommended by Musgrave.

Paramœba hominis (Craig).—Craig[2] observed organisms which apparently occupy a position intermediary between amebas and flagellates, in several cases of severe diarrhea occurring in the Philippine Islands. In one stage of its existence the parameba is capable of active progressive locomotion and is much larger than the trichomonas in the resting stage. In the flagellate stage it is distinguished from the corresponding stage of trichomonas by the absence of an undulating membrane, the presence of a single flagellum, and its circular form. The question of its pathogenicity has not been decided.

The *Flagellata s. mastigophora* differ from the rhizopoda in being provided with from one to eight flagella, which serve as organs of locomotion and possibly also for the apprehension of food particles. Representatives of two orders only, viz., the *monadina* and *isomastigoda*, have been found in the feces. Of the monadina in turn only one family, viz., the cenomonadina, and of the isomastigoda only two families, the tetramitina and polymastigina, are represented.[3]

The *cenomonadina* are small, oval, frequently elongated bodies, provided with one long flagellum at the anterior end, at the base of which food vacuoles are situated. At the posterior end ameboid movements may be observed, and there can be no doubt that the

[1] Amer. Med., 1905, pp. 850, 897, and 936.

[2] Amer. Jour. Med. Sci., August, 1906, p. 214.

[3] W. Janowski, Zeit. f. klin. Med., vol. xxxi, p. 445.

taking up of food, to some extent at least, also occurs by the aid of pseudopodia. To this family belongs the *cercomonas* of Davaine and Lambl. The *tetramitina* are small, elongated bodies, provided with four flagella and a lateral, undulating membrane, which was formerly mistaken for a posteriorly directed flagellum. The tail end of the organism tapers to a point. The nucleus is located at the base of the flagella. To this family belongs the parasite which was first discovered by Donné in the vagina, and which later was found also in the feces, and which has been variously designated as Trichomonas hominis, Cercomonas coli hominis, etc.

The *polymastigina* are small, somewhat oval bodies, provided with two or three flagella, situated either anteriorly or laterally—two or three on each side—while at the same time two additional flagella issue from the posterior end, which may either be rounded off or taper to a point. To this family belongs the Megastoma entericum of Grassi.

The question whether or not the flagellate bodies are of pathological importance still remains *sub judice*. They are apparently met with only in diseases associated with diarrhea, and it appears that in some cases at least this is directly dependent upon their presence; in others the impression is gained as though they merely maintained an already existing diarrhea referable to other causes; while in a third class of cases no relation can be discovered between their prescence and the disease in question. Cohnheim[1] has pointed out that living infusoria in the feces may be a symptom of a primary chronic stomach affection (gastritis, usually the atrophic form). According to the same writer, encysted infusoria may also be found in the feces of healthy individuals, but in such cases we may assume that at some time previously a gastritis or a gastro-enteritis has existed. He thinks they have no pathogenic significance, and are merely of symptomatic-diagnostic interest.

Cercomonas of Davaine-Lambl: *syn.*, Cercomonas hominis (Davaine); monas (Marchand); Monas lens (Grassi); Monas monomitina (Grassi). The adult organism (see Fig. 79) is oval or roundish in form, and provided anteriorly with a single long flagellum and posteriorly with a tail-like appendage. Its length varies from 0.005 to 0.014 mm. The younger forms are pear-shaped or S-shaped, and sometimes irregular in outline; the flagellum is then either absent or rudimentary.

Upon prolonged observation it will be seen that the adult parasite loses its flagellum and may protrude a protoplasmic process instead, while vacuolation occurs at the same time, indicating approaching death.[2]

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[1] Deutsch. med. Woch., 1903, vol. xxix, p. 248.

[2] Lambl, Prag. Vierteljahr., 1859, vol. lxi, p. 1. Davaine, Traité des entozoaires, 1860, Paris. Marchand, Virchow's Archiv, 1875, vol. lxiv, p. 293. Zunker, Deutsch. Arch. f. prakt. Med., 1878.

Trichomonas, Donné: *syn.*, Trichomonas vaginalis (Donné); Trichomonas hominis (Grassi); monocercomonas (Grassi); cimænomonas (Grassi); Protorycomyces coprinarius (Cunningham and Lewis); Cercomonas coli hominis (May); Trichomonas intestinalis (Leuckart

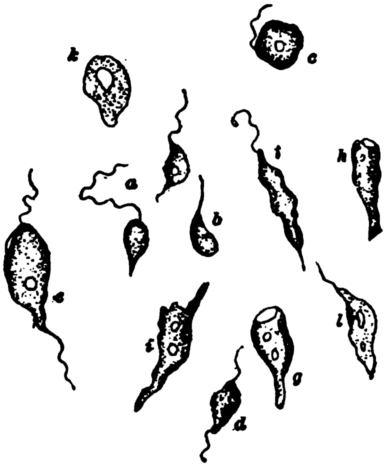

FIG. 79.—Cercomonas intestinalis: *a*, Cercomonas of Davaine, after Leuckart; *b*, Cercomonas intestinalis, after Lambl; *c*, *d*, same, ordinary forms: *e*, *f*, same, well-developed forms; *g*, *h*, *i*, same, degeneration forms; *k*, *l*, same, abortive forms.

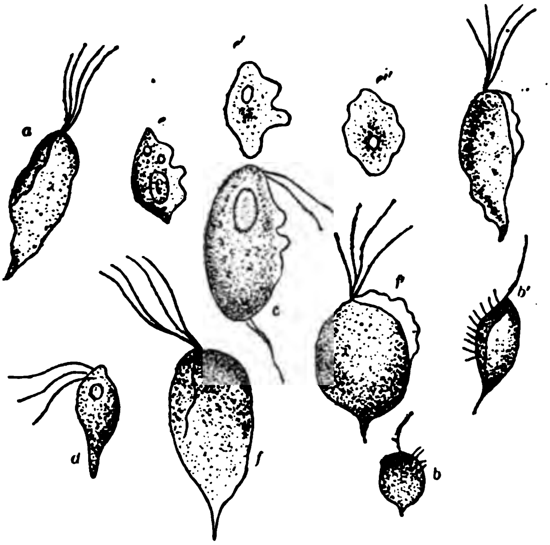

FIG. 80.—Trichomonas intestinalis: *a*, *a'*, *c*, trichomonas of the urine, after Marchand; *b*, Trichomonas vaginalis, after Donné; *d*, Trichomonas intestinalis, after Piccardi; *e*, *e'*, *e''*, same, ameboid forms; *f*, *f'*, trichomonas of the urine. (After Dock.)

and Roos); Cercomonas s. Bodo urinarius (Künstler). The parasite (Fig. 80) is oval or spindle-shaped and measures from 0.012 to 0.03 mm. in length by 0.01 to 0.015 mm. in breadth. From its anterior pole four flagella are given off, which are almost as long as the organism itself. From this point an undulating membrane extends laterally to the posterior pole, which may be rounded off or tapers to a tail-like appendage. This membrane is best seen when the movements of the flagella have ceased, as in specimens fixed in mercuric chloride solution (1 to 5000). The nucleus is situated at the base of the flagella, but is usually visible only in stained specimens (methylene blue). At times the organisms may be observed to assume an ameboid form; the movements of the flagella have then ceased, and pseudopodia-like processes are protruded. The parasite is identical with the trichomonas which has been found in the vagina and in the urine.[1] When present in the feces the organism is usually seen in large numbers. Not infrequently it is found associated with other intestinal parasites.

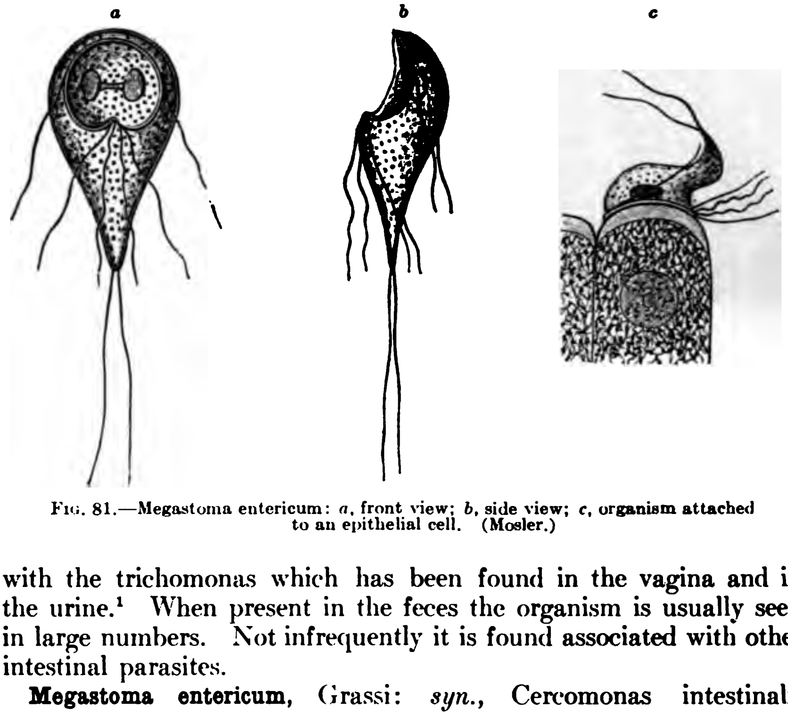

FIG. 81.—Megastoma entericum: *a*, front view; *b*, side view; *c*, organism attached to an epithelial cell. (Mosler.)

Megastoma entericum, Grassi: *syn.*, Cercomonas intestinalis (Lambl); Megastoma intestinale (Bütschli); Lamblia intestinalis (Blanchard); Dimorphus muris (Grassi). The parasite (Fig. 81) is pear-shaped, and measures from 0.01 to 0.021 mm. in length by 0.0075 to 0.05 mm. in breadth. In its anterior portion a more or less well-marked depression can be made out, which constitutes the peristome or mouth-opening of the organism. It is provided with eight flagella, grouped in pairs. The first pair originates on the sides of the peristome and is directed backward. The second and third pair are situated somewhat posteriorly and are likewise directed backward, while the fourth pair issues from the tapering tail end of the body. In fresh specimens the eighth flagella can usually not be made out, as the third and fourth pair are frequently agglutinated. The best results are obtained when the organism has been killed with mercuric chloride solution. The individual flagella vary from 0.009 to 0.014 mm. in length. In the anterior portion of the peristome two round, hyaline bodies can be recognized, which represent nuclei. Vacuoles are absent, and nutrition occurs through osmosis, the parasite adhering to epithelial cells by its periostome. When treated with fixing solutions the chitinous envelope can be readily recognized. In the encysted form the organism is oval and measures from 0.007 to 0.1 mm. in diameter.

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[1] Marchand, loc. cit. Zunker, loc. cit., p. 236. Mosler u. Peiper, Nothnagel's Spez. Path. u. Therap., 1894, vol. vi.

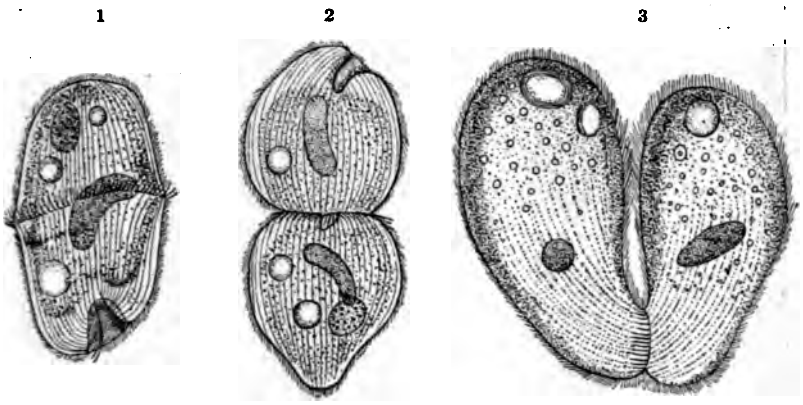

FIG. 82.—Balantidium coli: 1, 2, division; 3, conjugation. (After Leuckart, from Döflein.)

Grassi observed the organism in mice, rats, cats, dogs, rabbits, and sheep.[1] The *ciliata,* as the term indicates, carry cilia, and of these only one member, belonging to the *holotricha,* is found in the feces, namely, the Balantidium coli.

Balantidium coli, Stein: *syn.,* Paramœcium coli (Malmsten). The organism is oval and measures from 70 μ to 110 μ in length by 60 μ to 72 μ in breadth. It is covered entirely with fine, actively motile cilia, which are grouped most densely about the funnel-shaped mouth, while at the anus only a few are seen. An ectosarc and an endosarc may be distinguished, and the parasite possesses the power to change its shape, and may appear quite round. In its interior we find a large, somewhat kidney-shaped nucleus, two contractile vesicles, and frequently fat droplets, starch granules, etc. (Fig. 82).

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[1] Grassi u. Schewiakoff, Zeit. f. wiss. Zoologie, 1888, vol. xlvi, p. 143.

The parasite is probably pathogenic, but comparatively uncommon outside of Sweden, Finland, and Russia. Infection occurs through the dejecta of swine. Strong and Musgrave report that in their case blood examination showed a relative increase of the eosinophiles. From 200 to 300 organisms have been encountered in a single drop of the liquid feces.[1]

The fourth class of protozoa, viz., the *Gregarina* or *sporozoa*,[2] is also said to be represented in the human feces. The coccidia and psorosperms belong to this order. They are oval bodies, measuring about 0.022 mm. in length, and contain in their interior a large number of small nuclei arranged in groups. They are entirely devoid of organs of locomotion, and obtain their nutriment by endosmosis. Reproduction occurs in a common capsule, which bursts at a certain time and sends forth a whole generation of fully developed organisms. In human pathology they have become of interest in so far as certain observers have ascribed to them a role in the etiology of neoplasms. A disease of the liver analogous to the

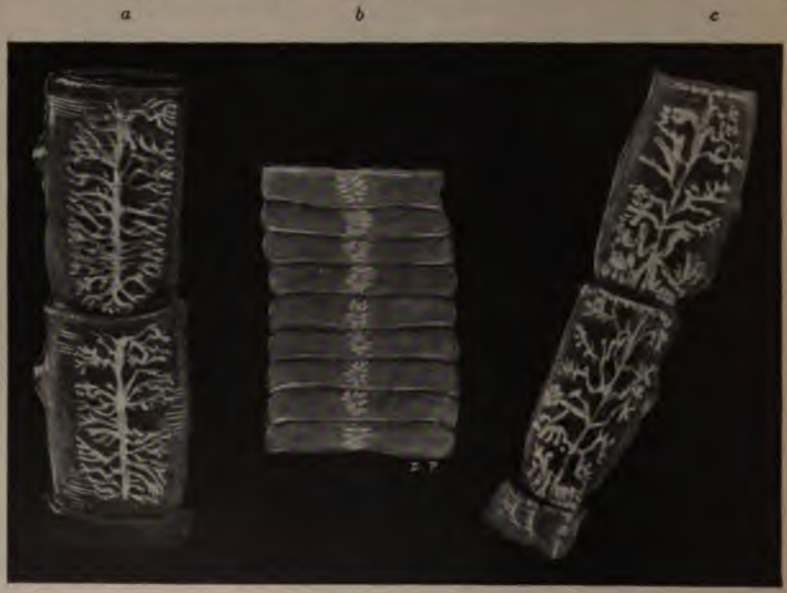

FIG. 83.—Segments of tapeworms: *a*, Tænia saginata; *b*, Bothriocephalus latus; *c*, Tænia solium.

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[1] Malmsten, Virchow's Archiv, 1897, vol. xii, p. 302. Sievers, "Ueber Balantidium Coli im menschlichen Darmkanal," Arch. f. Verdauungskrank., vol. v, p. 445. Janowski, "Balantidium Coli," Zeit. f. klin. Med., vol. xxxii, p. 303. Henschen, Arch. f. Verdauungsk., 1901, vol. vii, p. 501. Solorojew, Centralbl. f. Bacter., 1901, vol. xxix, pp. 821 and 849. A. Ehrenrooth, Zeit. f. klin. Med., 1903, vol. xlix, p. 321.

[2] v. Wasielewski, Sporozoenkunde, 1896.

psorospermiasis of rabbits has also been described in man, and parasites belonging to the same order have been observed in the skin.

Cestodes.—**Tænia saginata,** Goeze: *syn.*, T. mediocanellata (Küchenmeister); T. incruris (Huber); T. dentata (Nicola). This parasite (Fig. 84) is the most common tapeworm in Europe and North America.

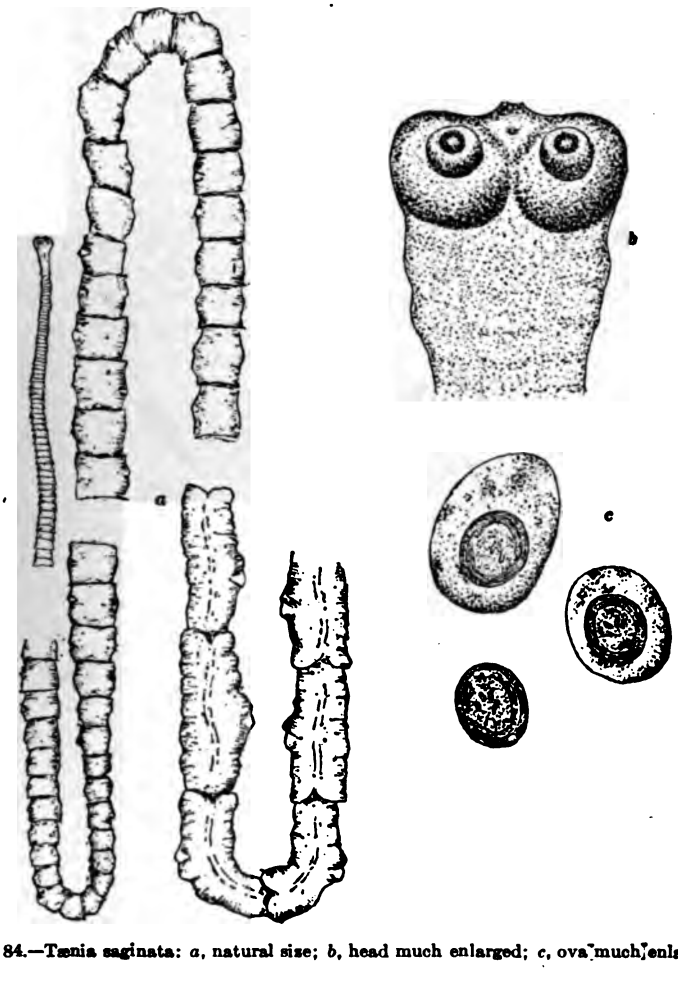

Fig. 84.—Tænia saginata: *a*, natural size; *b*, head much enlarged; *c*, ova much enlarged.

Infection occurs through the ingestion of measly beef. Its length varies from 4 to 8 m. The head, which is devoid of a rostellum, is surrounded by four pigmented suckers, each of which is encircled by a dark line. The individual segments are quite thick and opaque, and diminish in length as the head is approached, the largest measuring from 2 to 3 cm. They are each provided with a very much branched uterus, which opens laterally, the primary branches numbering about twenty on each side (Fig. 83). The ova are elliptical in form, of a brown color, and usually enclosed in a vitelline membrane (Plate XV). Upon careful observation a double contour with delicate, radiating striæ can be discerned. In the interior the booklets of the embryos, which are lost in the adult worm, are seen embedded in a brown, granular material.

The diagnosis is mostly made by the patient when segments are found in the stools. In doubtful cases the eggs should be looked for; they are readily seen with a low power (⅔ Bausch and Lomb).

The larval form of Tænia saginata, the so-called Cysticercus tæniæ saginatæ (Leuckart), or the Cysticercus bovis (Cobbold), has been encountered in cattle, the Rocky Mountain "antelope," the llama, and the giraffe. In the human being it has not been observed.[1]

Tænia solium, Rudolphi: *syn.*, T. cucurbitina, plana, pellucida, Goeze. This parasite (Fig. 85) is far less common in this country than the Tenia saginata, and may indeed be regarded as a curiosity. In Germany, also, it is only rarely met with now, while formerly it was the most common tapeworm in that country. This change is undoubtedly owing to the fact that raw pork is now eaten less frequently. In Asia and Africa it is more common.

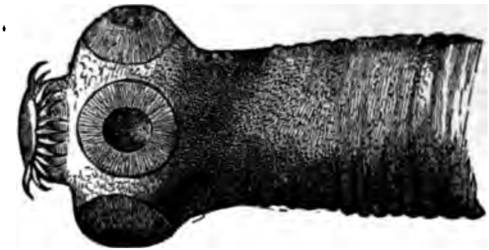

FIG. 85.—Head of T. solium. × 45. (Leuckart.)

Tænia solium is usually much shorter than Tenia saginata, rarely exceeding 3.5 m. in length. Most characteristic is the head, which is provided with four pigmented suckers and a rostellum, furnished with from twenty-four to twenty-six hooklets arranged in a double row. The mature segments measure from 1 to 1.5 cm. in length by 6 to 7 mm. in breadth, and contain a uterus which has only five to seven branches, thus differing greatly from that of Tænia saginata. The ova are round, of a brownish color, and surrounded with a thick, radially striated membrane; in their interior the hooklets of the embryos can usually be made out. They are readily found in the feces and should be looked for in doubtful cases.

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[1] J. Ch. Huber, Die Darmcestoden des Menschen. Bibliograph. d. klin. Helminthol., Heft 3, No. 4, p. 69, München, 1892. R. Leuckart, Die Parasiten des Menschen, etc., 2d ed., 1880, pt. i.

The larval form of this tapeworm, the *Cysticercus cellulosæ*, has been found in swine, the wild boar, in monkeys, in the brown bear, in the dog, etc. At times, though rarely, an auto-infection with the proglottides of Tænia solium has also been observed in the human being. Under such conditions the embryos of the worm are set free in the stomach, and may then migrate into various parts of the body, where they become encysted. Most commonly the cysticerci are found in the skin; they have, however, also been observed in the heart, the lymph glands, liver, bones, tongue, spinal canal, the brain and the eyes. I have had occasion to observe a case of this kind at the Johns Hopkins Hospital (reported by Osler). The patient, a laboring man, had never worked as a butcher or a cook, and never had a tapeworm. The cysticercus nodules, which were situated between the skin and the fascia, were very numerous, seventy-five being counted on one day. One of these nodules was removed for examination, and was shown to be referable to the cysticercus of Tænia solium. The only subjective complaints in this case were pains and stiffness in the arms and legs. The individual cysticercus was elliptical or roundish in form, measuring from 1 to 10 mm. in diameter. In its interior the characteristic hooklets were seen.[1]

Tænia nana, v. Siebold: *syn.*, hymenolepis (Weinland). This parasite (Fig. 86) seems to be the most common tapeworm of Italy and Egypt. It has also been seen in Buenos Ayres, in Bangkok, Siam, and a few isolated cases have been reported in England and in Germany. In the United States the parasite seems to be not at all uncommon, but has probably been overlooked in many cases. Stiles states that in his laboratory eighteen cases have been diagnosticated within a year (1902). It is found especially in young people, and often causes severe nervous symptoms. It is only 8 to 25 mm. long and 0.5 mm. broad. The head is ball-shaped and provided with four suckers and a rostellum, bearing twenty-four to twenty-eight hooklets arranged in a single row along its anterior edge. The individual segments are of a yellowish color and about four times as broad as long. The uterus is oblong and contains numerous ova, which are colorless, oval, and surrounded by a distinct, non-striated membrane. They measure from 0.839 to 0.060 mm. in size. In their interior the embryonic worm, provided with five or six hooklets, may be distinguished. The number of worms which may at times be found in the digestive tract is most astonishing; 5000 and even more have been counted on several occasions. The cysticercus stage occurs in snails, which are frequently eaten raw in Egypt and Italy. Tænia nana has been identified with the Tænia murina of rats and

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[1] Huber, loc. cit. Leuckart, loc. cit.; and Blanchard, Traité de Zoologie médicale, vol. iv, Paris. The Inspection of Meats for Parasites, Bull. No. 19, Bureau of Animal Industry, Washington, 1898.

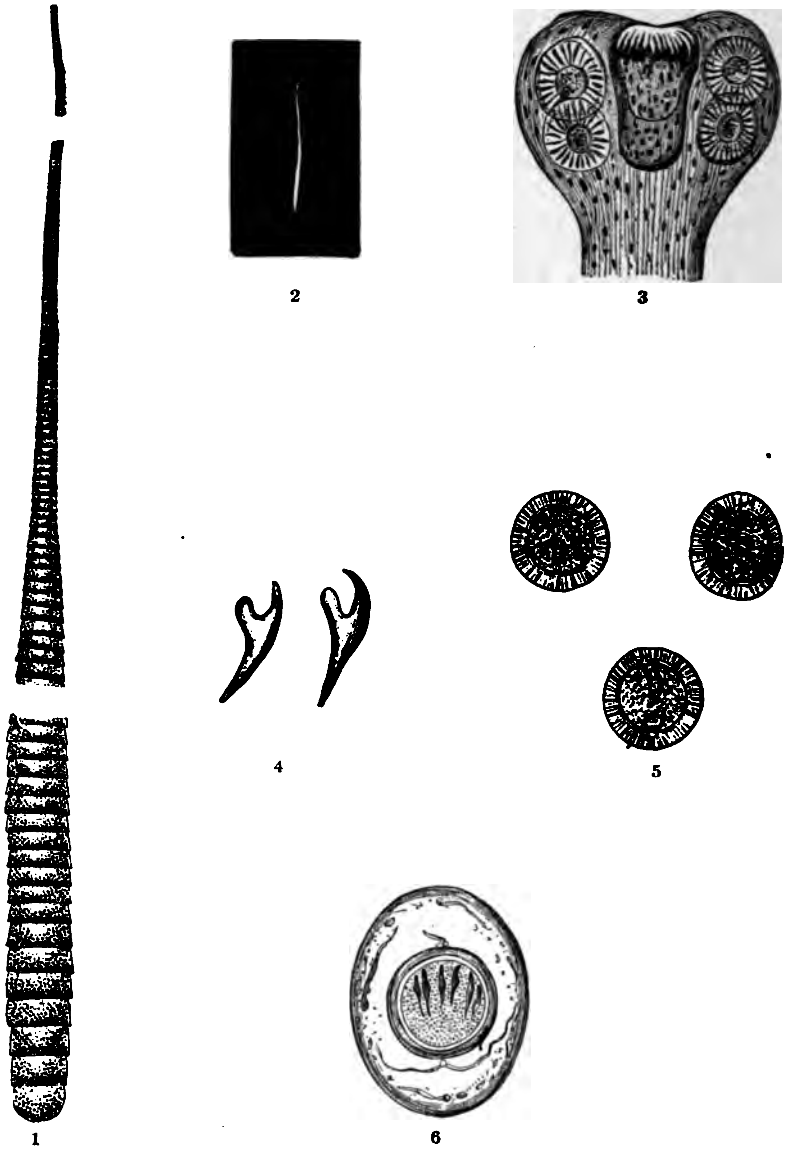

Fig. 86.—Tænia nana: 1, body; 2, natural size; 3, head; 4, hooklets; 5, eggs; 6, egg magnified 600 times. (From Mosler.)

other rodents.[1] In doubtful cases the eggs should be looked for; they are readily seen with a low power (B. and L. $\frac{2}{3}$).

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[1] Grassi, Centralbl. f. Bakt. u. Parasit., 1887, vol. i, p. 97. Grassi u. Calandruccio, ibid., 1887, vol. ii, p. 282. Comini, ibid., p. 27. Bilharz, cited by Leuckart. C W Stiles, New York Med. Jour., Nov. 7, 1903.

Tænia diminuta, Rudolphi: *syn.*, Tænia flavapunctata (Weinland); **ænia minima** (Grassi); Tænia varerina (Parona); Tænia leptoephala (Creplin). Tænia diminuta was first described in man by eidy, Grassi, and Parona. It measures 20 to 60 mm. in length, nd is armed with two suckers, but is without a rostellum. The va resemble those of Tænia solium. The cysticercus occurs in ertain caterpillars and cocoons. In man it has been found in only ix instances.[1]

Dipylidium caninum, Linné: *syn.*, Tænia canina (Linné); Tænia noniliformis (Pallas); Tænia cucumerina (Bloch); Tænia elliptica Batsch). The parasite is found almost exclusively in children; nfection occurs through dogs and cats. In the United States the lisease is apparently rare. The only case reported is that of Stiles.[2] The larval form is found in lice and fleas. The worm itself measures rom 15 to 35 cm. in length. The head is small, globular; the rostelum club-shaped with 3 or 4 transverse rows of hooks (about 60 n number) of rose-thorn form; anterior hooks 15 μ, posterior hooks 6 μ; suckers relatively large, rather elliptical. Segments 80 to 120 n number; gravid segments 8 to 11 mm. long, 1.5 to 3 mm. broad; often reddish-brown in color. Genital pores at equator or in posterior half of segment; uterus forms egg capsules, each containing from 8 o 20 eggs, eggs globular, 43 to 50 μ in diameter. The ova contain embryos already armed with hooklets (Stiles). In diagnosis Stiles suggests that search be made in the feces for the peculiar elongated elliptical tapeworm segments (Fig. 87). Microscopic examination of the feces for eggs is less certain than in cases of infection with Tænia saginata, Tænia solium, or Dibothriocephalus latus, since Dipylidium is much smaller and less prolific than any of these three orms.[3]

Tænia Africana, v. Linstow.[4]—This parasite has been found in two nstances, in the case of two native soldiers at Nyasa Lake. Like he scolex of Tænia saginata, that of the present species is devoid of hooklets. Its length is about 1.4 m.; the number of segments about 00. They are all much broader than long. The uterus consists of a main portion running fore and aft, from which from 15 to 24 side branches issue, which do not branch dichotomously and are so closely packed that they cannot be recognized with the naked eye.

Tænia Madagascariensis (Grenet).—This parasite has been found in Madagascar, in Mauritius, in Bangkok, and in a Demarara Indian. The worm attains a length of from 25 to 30 cm. and is composed of

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[1] Leidy and Parona, cited by Leuckart.

[2] Amer. Med., 1902, vol. v, p. 65.

[3] A. Hoffmann, Jahresb. f. Kinderheilk., 1887, vol. xxvi, Heft 3 u. 4. Krüger, St. Petersburg. med. Woch., 1887, vol. xii, p. 341. Brandt. Centralbl. f. Bakt. u. Parasit., 1889, vol. v, p. 99.

[4] Centralbl. f. Bakt. u. Parasit., 1900, vol. xxviii, p. 485.

from 500 to 600 trapezoid segments. The rostellum is surrounded by a double row of minute hooklets. The suckers are round and quite large. Blanchard suggests that the cockroach may be its intermediary host.

Dibothriocephalus latus, Linné, Lueke: *syn.*, Bothriocephalus latus, (Bremser), Tænia lata (Linné); Dibothrium latum (Rudophi) (see Fig. 88). This worm is usually 5 to 10 m. long and of a reddish-gray color. Longer specimens, however, may also be encountered. In Wilson's case 82 feet of segments were obtained from two worms, so that the length of each, supposing both to have been of the same size, must have been more than 40 feet. The head is almond-shaped

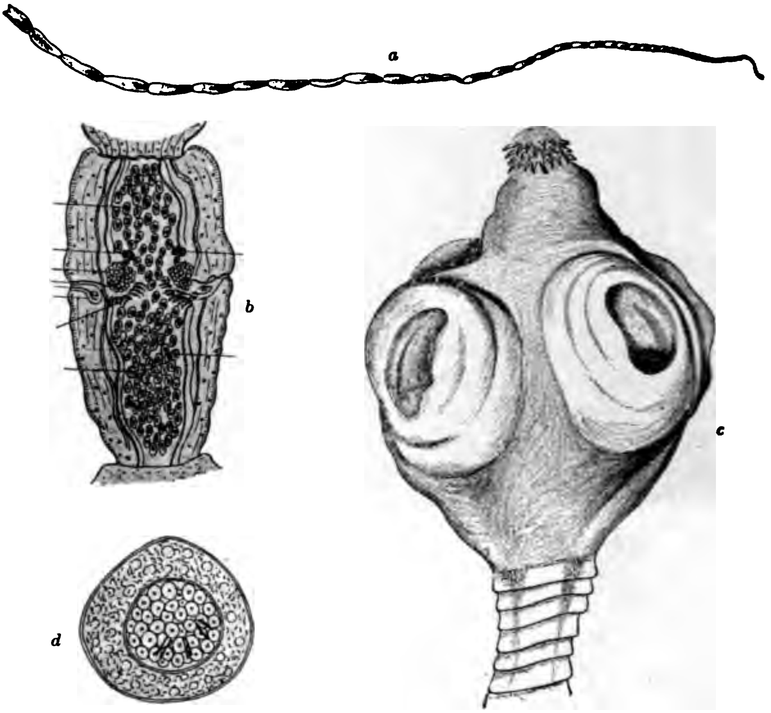

FIG. 87.—*a*, Dipylidium caninum (taken from Stiles); *b*, gravid segment (after Diamare); *c*, head, showing four rows of rose-thorn hooks on the rostellum and four unarmed suckers (Stiles); *d*, egg, showing six hooks of the embyro (Stiles).

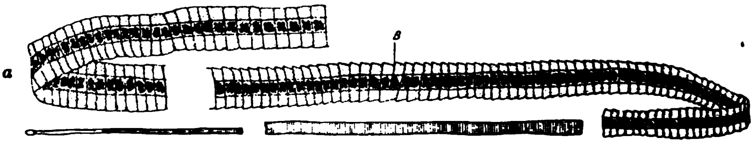

FIG. 88.—Bothriocephalus latus: *a*, *b*, twin segments. (Wilson.)

and upon its flat surfaces two distinct grooves can be discerned, which probably act as suckers. It measures 2 to 3 mm. in length by 1 mm. in breadth. The neck is very short and passes at once into the body segments. Adjacent segments can often be distinguished only by means of the recurrence of the sexual apparatus, which appears regularly in spite of the imperfect individualization of the segments. The ripe segments are almost square in form, with the genital apparatus opening in the median line. The fully developed segments measure 2.5 to 4.5 mm. in length by 8 to 14 mm. in breadth. The total number of segments may far exceed 3000. The frequent occurrence of imperfect and abortive types of twin segments may be considered an almost distinctive feature of the bothriocephalus family (Wilson). The uterus presents 4 to 6 convolutions on each side, which become especially distinct when the segments are placed in water or are exposed to the air. A rosette-like appearance is then noted, which is quite characteristic (Fig. 83). The rosette deepens in color in proportion to the number of ova which the uterus contains, and toward the tail of the parasite, from the segments of which many or all the eggs have been discharged, the rosette tends to become light in color, and may indeed appear whiter than the surrounding parenchyma. The eggs (Fig. 89) are oval, 0.06 to 0.07 mm. long and about 0.045 mm. broad; they are enclosed in a brown envelope, at the anterior end of which a little lid can be recognized. Their contents consist of protoplasmic spherules, all of about the same size, which are lighter in color in the centre than at the periphery. In infected individuals they are constantly found in the stools.

The larvæ have been found in various fresh-water fishes, such as the perch, the ling, the turbot, in various members of the trout family, but they are most commonly encountered in the pike. It is thus readily understood why the parasite is most common in lake regions, as in Switzerland, northern Russia, southern Scandinavia, and northern Italy. It is seldom seen in middle Germany, but is so common in Ireland that Cobbold named it the Irish tapeworm. Outside of Europe it is most common in Japan. In the United States a few imported cases have been observed by Walker and Leidy, Packard, Hageestam, Riesman, Stengel, McFarland, and Wilson.

Multiple infection has been repeatedly observed. Böttcher notes a case in which 100 worms were found; Roux and Eichhorst both speak of cases with 90, Heller of one with 38, and in Wilson's case 2 were undoubtedly present. When more than 1 occurs the growth of the individual is impeded, and small specimens are then usually seen (three to five feet or more). Clinically the parasite is of especial interest, as its presence in a certain percentage of cases is associated with the clinical picture of a pernicious anemia; in others, however, no deleterious effect upon the red corpuscles is noted, although several worms may be present in the intestinal tract.

Besides in man, the worm has been encountered in the dog, cat, the seal, and in some water birds. The ovum, after being discharged in the feces, during a variable period of incubation in the water develops into the onchosphera, a ciliated larva with six hooklets (Fig. 91). The larva is then liberated from the ovum by passing through the lidded end, and by means of its cilia moves rapidly through the water. If not eaten by fish, it dies; otherwise it develops into the bothriocephalus measle, the plerocercoid (Fig. 90), which has both head and tail. Infection of man then occurs when such fish are eaten either raw or but partly cooked. In man the cysticercus stage has not been observed.[1]

Krabbea grandis, Blanchard.—This parasite has been observed in only one instance—in Japan. It is said to resemble certain bothriocephali which are found in seals. The genital organs are double in each segment. The vulva and uterus open ventrally. The worm attains a length of 10 m. with a breadth of 2 cm.

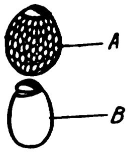

FIG. 89.

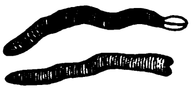

FIG. 90.

FIGS. 89 and 90.—Eggs and plerocercoid. (Braun.)

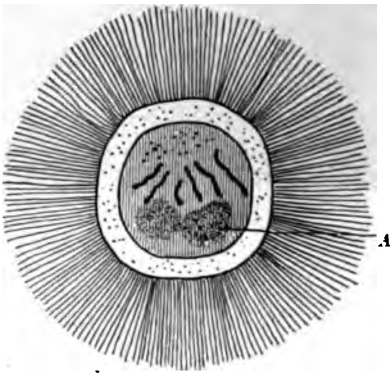

FIG. 91.—Embryo with cilia and hooklets of Bothriocephalus latus. (Leuckart and Braun.)

Trematodes.—The various forms of distoma which belong to this order are essentially hepatic parasites, and rarely occur in the feces.

Distoma hepaticum, Abildgaard: *syn.*, Fasciola hepatica (Linné) (Fig. 92). This, the most common liver fluke, is 28 mm. long and 12 mm. broad; it is formed like a leaf. The leaf is provided with a sucker, and a second sucker may be found at its ventral surface. Between the two the genital opening is located, leading into a skein-shaped uterus. The eggs are oval, measuring 0.13 mm. in length and 0.08 mm. in breadth, the anterior end being provided with a lid; their color is brown. In the United States the organism is practically unknown, while in Germany it is most common in sheep. In the human being it is rare in both countries. It occurs in cattle, sheep, swine, cats, rabbits, etc. Infection occurs through a small snail, the Linnæus minutus, which is found, in Germany especially, upon watercress.[1]

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[1] Schaumann, Zur Kenntniss d. sogenannten Bothriocephalus-Anaemie, Berlin, 1894. Schaumann u. Tallqvist, "Ueber d. blutkörperchenauflösenden Eigenschaften d. breiten Bandwurms," Deutsch. med. Woch., 1898, p. 312. Runeberg, Deutsch. Arch. f. klin. Med., 1887, vol. xli, p. 304. Askanazy, Zeit. f. klin. Med., 1895, vol. xxvii, p. 492. R. N. Wilson, "Bothriocephalus, Report of a Case of Double Infection," Amer. Jour Med. Sci., 1902, vol. cxxiv, p 262.

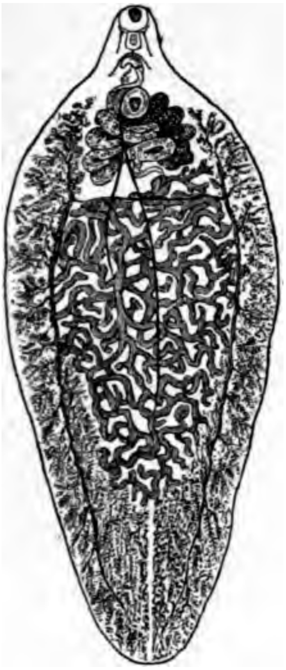

Fig. 92.—Distoma hepaticum, with male and female genital apparatus. (From Ziegler, after Leuckart.)

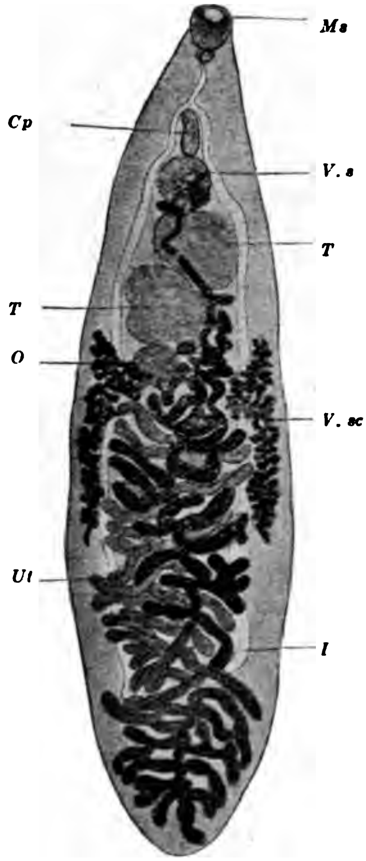

Fig. 93.—Dicrocœlium (Distoma) lanceolatum, Stil. and Hass; *V. s.*, ventral sucker; *Cp*, pouch of cirrus; *I*, intestinal furcations; *V. sc.*, vitelline sacs; *T*, testicles; *O*, ovarium; *Ms*, oval sucker; *Ut*, uterus.

Distoma lanceolatum, Mehlis, has been found in only five cases, all of which occurred in Germany (Fig. 93). It is much smaller than Distoma hepaticum, measuring 8 to 9 mm. in length, by 2 to

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[1] C. W. Stiles, Jour. Comp. Med. and Vet. Arch., 1894, vol. xv, and 1895, vol. xvi. Huber, Trematoden, Bibliog. d. klin. Helminthol., Heft 7 u. 8, p. 283.

3.3 mm. in breadth. It is lancet-shaped, tapering toward the head end, but otherwise closely resembles Distoma hepaticum. The ova are 0.04 mm. long, 0.03 mm. broad, and contain fully developed embryos. In cattle, sheep, and hogs the organism is quite common.[1]

Distoma Buski, Lankester: *syn.*, Distoma rhatonisii (Poirier); Distoma cranum (Busk); Fasciolopsis Buski (Lankester.) The parasite has been observed in China, Sumatra, the Straits Settlements, Assam, and India. An imported case has been described in the United States (Moore). It is the largest distoma occurring in man, measuring over an inch in length. It probably inhabits the upper portion of the intestine and may give rise to attacks of recurring diarrhea and other signs of intestinal irritation. Infection probably occurs through certain fishes and oysters, with certain snails as intermediary hosts.[2]

Distoma sibiricum, Winigradoff: *syn.*, Distoma felinum (Rivolta). This parasite was found in Tomsk, by Winigradoff, in eight autopsies out of one hundred and twenty-four. Askanazy also reports two cases of infection from eastern Prussia, in which the eggs were found in the stools. In one of the cases, which came to section, more than one hundred organisms were found in the biliary passages. Its length may reach 13 mm. The ova are 0.026 to 0.038 mm. long and 0.010 to 0.022 mm. broad. The intestine is simple and extends to the posterior extremity of the body. Its surface is smooth.[3]

Distoma spatulatum, Leuckart: *syn.*, Distoma sinense (Cobbold); Distoma endemicum (Balz); Distoma japonicum (Blanchard). It has been observed in India, Mauritius, Corea, Formosa, China, Tonkin, and Japan, and it appears that in the two last-named countries it is quite common. It inhabits the biliary passages and gall-bladder. It is distinctly pathogenic. The ova may be found in the stools. The parasite possibly also occurs in cats. The intermediary host is not definitely known; it may be some fresh-water mollusk. It is about 11.75 mm. long and 2 to 2.75 mm. broad. The living parasite is of a reddish color and translucent, so that it is possible to distinguish all its interior organs. The ova measure 0.028 to 0.030 mm. in length by 0.016 to 0.017 mm. in breadth, and are enclosed in a colorless envelope.[4]

Other parasites belonging to this order are **Distoma conjunctum** (Cobbold), **Distoma heterophyes** (v. Siebold), and **Amphistomum hominis** (Lewis and McConnell). The last named appears to be common in elephants and has been encountered in natives of Assam, in two Indians in Calcutta, and in an East Indian immigrant in

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[1] Leuckart, loc. cit., p. 137.

[2] Poirier, Centralbl. f. Bakt. u. Parasit., 1888, vol. ii, p. 186.

[3] Winigradoff, cited by Braun, Centralbl. f. Bakt. u. Parasit., 1894. vol. xv, p. 602.

[4] Blanchard, loc. cit.

British Guiana. It is quite small, measuring from 5 to 8 mm. in length by 3 to 4 mm. in breadth and is characterized by the large size of its posterior suckers.

Distoma heterophyes is the smallest distoma, so far as we know, which is found in man. It occurs in Egypt and is thought to be innocuous. (Fig. 94.)

Distoma conjunctum was discovered in an East Indian. Its surface is covered with minute spicules. It is not of much pathological importance. (Fig. 95.)

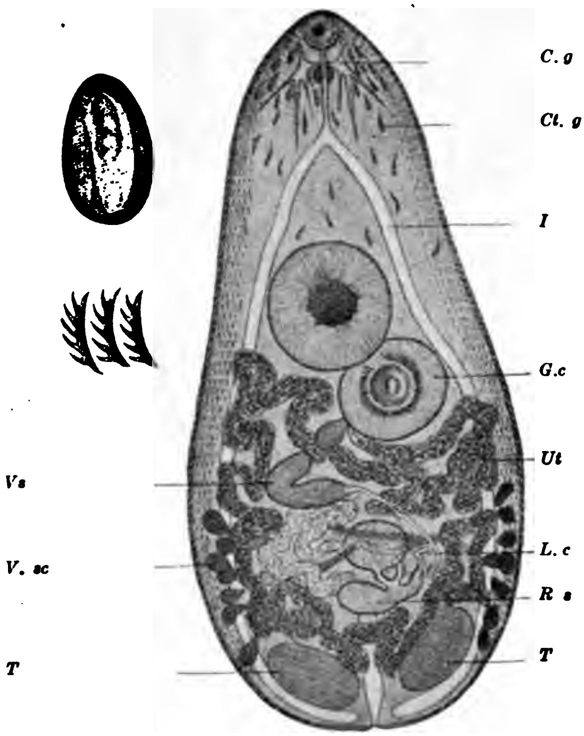

FIG. 94.—Cotylogonimus (Distoma) heterophyes. × 53 (v. Sieb.); *C. g*, cerebral ganglion; *I*, intestinal branches; *Ct. g*, cuticular glands; *V. sc*, vitelline sacs; *G. c*, genital cup; *T*, testes, the excretory bladder between them; *L. c*, Laurer's canal; *R. s*, receptaculum seminis, with the ovarium in front of it; *Ut*, uterus; *Vs*, vesicula seminalis. On the left side above, an egg × 700 is depicted, and below it three chitinous rodlets from the genital cup. × 700. (Looss.)

Distoma hæmatobium and **Distoma pulmonale** are described in the sections on the Blood and the Sputum, respectively.

Annelides.—The annelides are very common intestinal parasites, and of these especially the *nematodes.*

Ascaris lumbricoides, Linné (Fig. 96), is the cylindrically shaped worm so commonly seen in children and in the insane. The head consists of three projections or lips, which are provided with suckers

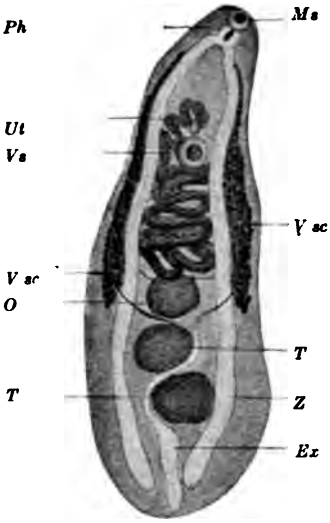

FIG. 95.—Distoma conjunctum, Cobb (*nec* Lewis and Crum; *nec* McConnell), from *Canis fulvus* (Cobbold): *Vs*, ventral sucker; *I*, intestine; *V sc*, vitelline sacs; *Ex*, excretory bladder; *T*, testes; *O*, ovary; *Ms*, oral sucker; *Ph*, pharynx; *Ut*, uterus.

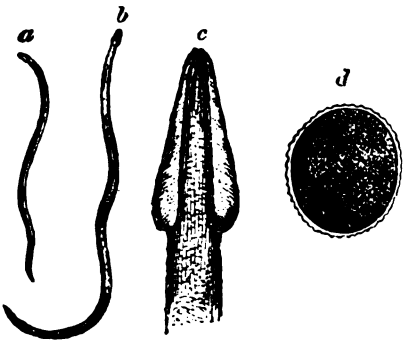

FIG. 97.—Ascaris mystax. (v. Jaksch.) *a*, male; *b*, female; *c*, head; *d*, egg.

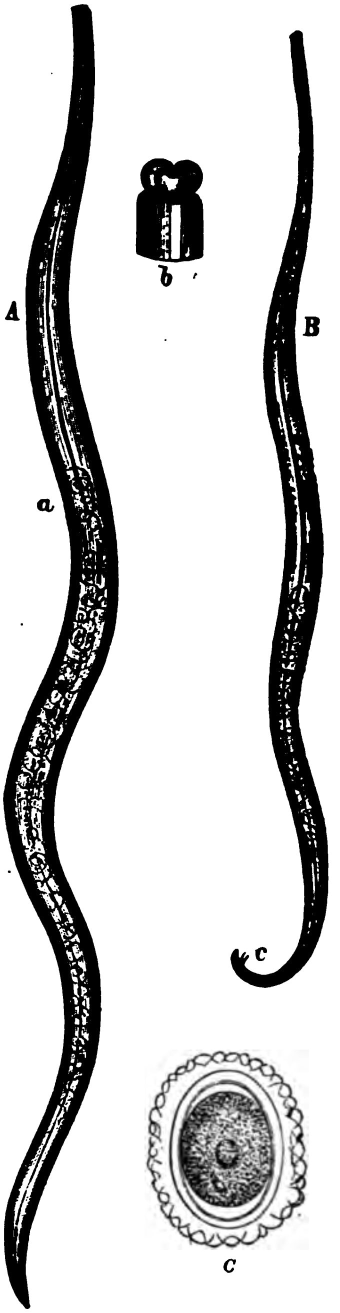

FIG. 96.—Ascaris lumbricoides: *A*, female; *B*, male; *C*, egg; at *a* the female genital opening; *c*, the male spicules; *b*, the enlarged cephalic extremity, with its three lips. (After Perlo, from Ziegler.)

and fine teeth. The male measures about 215 mm., the female about 400 mm. in length. The tail end of the male is rolled up on its ventral surface like a hook, and is provided with papillæ. The genital aperture of the female is situated directly behind the anterior third of the body. The eggs are yellowish brown in color, almost round, and measure 0.06 mm. by 0.07 mm. in size; they are surrounded by an irregular albuminous envelope, which is covered by a tough shell; the contents are coarsely granular.

Ascaris lumbricoides is found in all countries, and also infests the pig and the ox. Its presence may occasion severe nervous symptoms.[1]

Ascaris mystax, Zeder: *syn.*, Ascaris marginata (Rudolphi); Ascaris alata (Bellingham) (Fig. 97). This worm is smaller and thinner than Ascaris lumbricoides, but otherwise very similar. The head is pointed and provided with wing-like projections which constitute the main point of difference between the two. The male measures 45 to 60 mm. in length, the female 110 to 120 mm. Its ova are round, larger than those of Ascaris lumbricoides, and enclosed in a membrane which is covered with numerous small depressions. The worm is common in dogs and cats, but very rare in man.[2]

Ascaris maritima, Leuckart, also belongs to this class. It has been observed in only one case—in Greenland.

Ascaris Texana (Smith-Goeth).[3] A supposedly new species, which has been found in a single instance in Texas. The male has not yet been described.

Oxyuris vermicularis, Bremser: *syn.*, Ascaris vermicularis (Linné); Ascaris græcorum (Pallas) (Figs. 98, 99, and 100). The male is 4 mm., the female 10 mm. long. At the head three lip-like projections with lateral cuticular thickenings may be seen. The tail of the male is provided with six pairs of papillæ and the female with two uteri. The eggs are 0.05 by 0.02 to 0.03 mm. in size, and covered with a membrane showing a double or triple contour; in the interior, which is coarsely granular, the embryos are contained.

The female worm lives in the cecum, but after impregnation travels downward to the rectum. Here it causes most annoying symptoms, which are especially distressing at night, when the organism emerges from the anus. In doubtful cases of pruritus ani et vulvæ an examination of the feces should be made for this parasite. The ova themselves do not occur in the feces.[4]

Uncinaria duodenalis (Roilliet), *Ankylostomum duodenale* (Dubini): *syn.*, Ankylostoma duodenale (Dubini); Strongylus quadridentatus

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[1] Lutz, Centralbl. f. Bakt. u. Parasit., 1888, vol. iii, pp. 553, 584, 616. Hogg, Brit. Med. Jour., 1888, p. 121. Kartulis, Centralbl. f. Bakt. u. Parasit., vol. I. p. 65.

[2] K. A. Rudolphi, Arch. f. Zool. u. Zoot., 1803, vol. iii, pt. ii, p. 1. Idem, Entozoorum s. vermium intestinal. historia naturalis, Amsteraedami, ii, 2.

[3] Jour. Amer. Med. Assoc., Aug. 20, 1904, p. 542.

[4] Lutz, loc. cit.

(v. Siebold), Dochmius ankylostomum (Molin); Sclerastoma duodenale (Cobbold); Strongylus duodenalis (Schneider); Dochmius duodenale (Leuckart) (Figs. 101 to 103). This organism belongs to the family *Strongyloides*, and is one of the most dangerous parasites met with in the human being. It has been found in Italy, Germany, Switzerland, Belgium, Egypt, and the West Indies. C. W. Stiles has shown that a distinct species of the hookworm exists in the United States as also in the West Indies, viz., in Cuba and Porto Rico, the *Uncinaria Americana*, and that in the sand regions of the South infection with this parasite is common. Infection occurs very largely through the skin and perhaps altogether so. C. A. Smith insists that uncinariasis exists in all cases in which ground itch has occurred

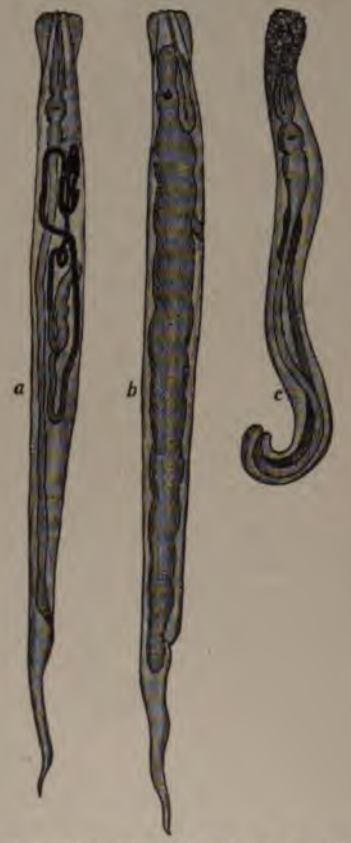

FIG. 98.—Oxyuris vermicularis: *a*, sexually mature female; *b*, female filled with eggs; *c*, male. Magnification, 10. (After Heller, from Ziegler.)

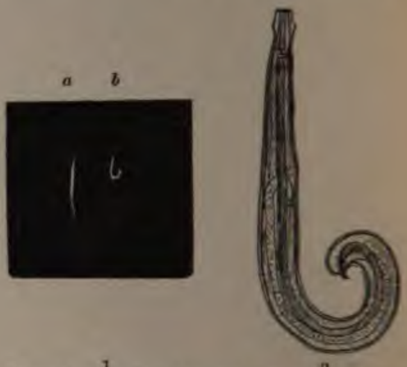

FIG. 99.—1. Oxyuris vermicularis: *a*, male; *b*, female; natural size. 2. Magnified.

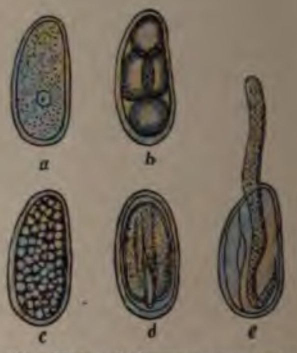

FIG. 100.—Eggs of Oxyuris vermicularis in various stages of development: *a*, *b*, *c*, division of the yolk; *d*, tadpole-like embryo; *e*, worm-shaped embryo. Magnification, 250. (After Zenker and Heller, from Ziegler.)

FIG. 102.—Ankylostoma duodenale, male and female. Natural size. (From Mosler.)

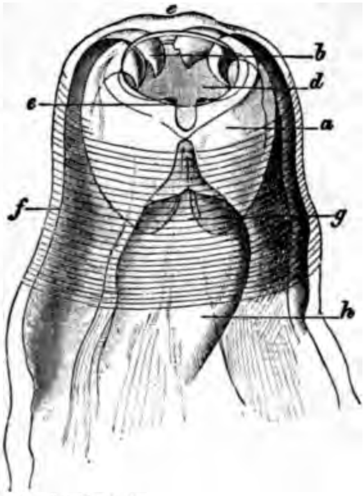

FIG. 103.—Head of Ankylostoma duodenale: *a*, buccal capsule; *b*, teeth of capsule; *c*, teeth of dorsal margin; *d*, oral cavity; *e*, ventral prominence; *f*, muscle layer; *g*, dorsal groove; *h*, esophagus. (After Schulthess, from Ziegler.)

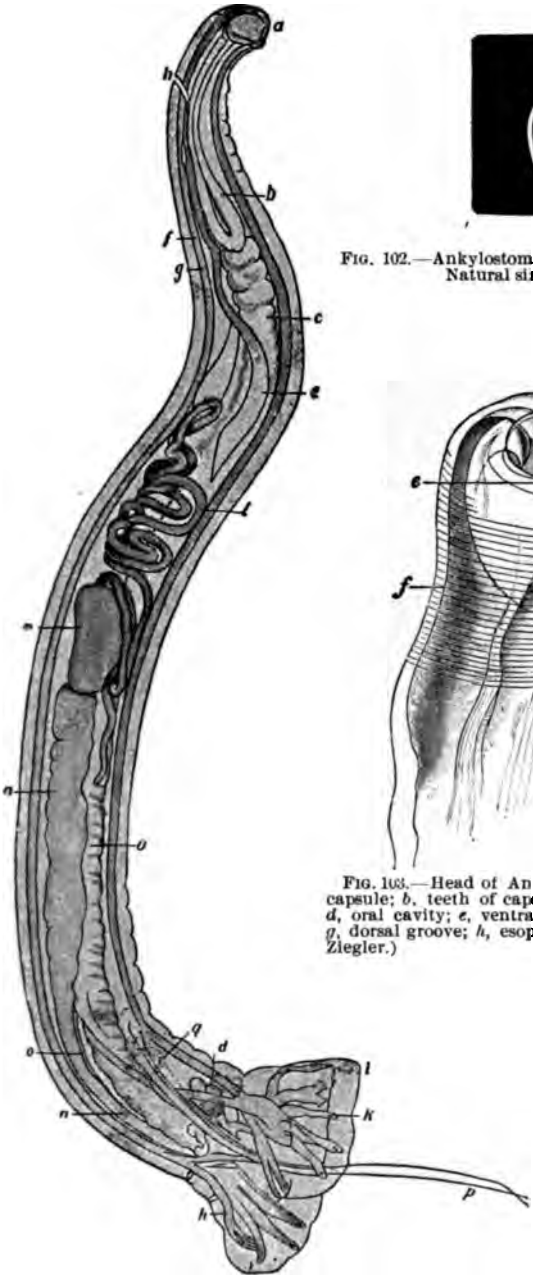

FIG. 101.—Male Ankylostoma duodenale: *a*, head: *b*, esophagus; *c*, gut; *d*, anal glands; *e*, cervical glands; *f*, skin; *g*, muscular layer; *h* excretory pore; *i*, trilobed bursa; *k*, ribs of bursa; *l*, seminal duct; *m*, vesicula seminalis; *n*, ductus ejaculatorius; *o*, its groove; *p*, penis; *q*, penile sheath. Magnification, 20. (After Schulthess, from Ziegler.)

within eight years, and that the disease is rarely if ever present in those who have not had ground itch within that time.

From a pathological standpoint the parasite is of special interest, as its presence may give rise to severe and fatal anemia. Griesinger was the first to point out that the so-called Egyptian chlorosis is produced by this organism. Subsequently it was shown that the same parasite was responsible for the anemia which developed among the workers on the St. Gothard tunnel, and which is common among the brickmakers in certain districts in Germany. In this country the anemia of the dirt-eaters has long been known in the South, and has been generally attributed to the peculiar habit. Its real cause is now manifest. In Porto Rico the disease was very common until very recently and responsible for much of the severe anemia which was so frequent among the natives. In Germany, France, and Belgium the mining districts have become extensively infested and the eradication of the disease a serious problem.

Outside of man the parasite is not uncommon in dogs, cattle, and sheep.

The male is 6 to 11.5 mm. long, the female 10 to 18 mm. The head, which tapers somewhat, is turned toward the back; the mouth capsule is hollowed out and surrounded by 4 teeth;[1] the tail of the male forms a 3-lobed bursa, while that of the female tapers conically; the genital opening is behind the middle of the body. Its eggs have an oval form and a smooth surface, measuring from 0.05 to 0.06 by 0.03 to 0.04 mm. In their interior two or three segmenting bodies are found, which rapidly develop outside of the human body, so that after twenty-four to forty-eight hours embryos may be found in the same feces in which the eggs were observed, or fully developed ova may be found after allowing the feces to stand for only a few hours (Plate XV). When allowed to dry, the young parasites become encysted, but after remaining so even for from one to two weeks they are capable of infection. A second host for its cycle of development is, according to Leichtenstern, not necessary.[2]

The habitat of the adult worm is the jejunum. It is rarely found in the feces. Its eggs, however, are common, and should be looked for in every case of anemia the cause of which is not manifest, especially in miners, tunnel-workers, brickmakers, dirt-eaters, etc. Any specimen of fecal material will answer as a rule, but it is best to procure a thin stool, as after a purge. It is then merely necessary to mount a small drop on a slide and to examine the covered specimen with a low power; a Bausch & Lomb ⅔ is quite sufficient. A mental picture of the size of the eggs should be made, for I have known it to occur that an observer saw the eggs, but did not recognize them as such. Once seen, they are easily recognized again.

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[1] The American species has only one dorsal, conical tooth, which projects prominently into the buccal cavity (Stiles).

[2] Centralbl. f. klin. Med., 1885, vol. vi, p. 195; Deutsch. med. Woch., 1885, vol. xi; 1886, vol. xii; 1887, vol. xiii. Lutz, Volkmann's Sammlung, 1885, Nos. 255 and 256. American cases: C. W. Stiles, "The Significance of the Recent American Cases of Hookworm Disease," Eighteenth Annual Report of Bureau of Animal Industry, 1901. H. F. Harris, Amer. Med., Nov. 15, 1902, p. 776. A. J. Smith, Am. Jour. Med. Sci., 1903, vol. cxxvi, p. 768. C. F. Craig, ibid., p. 798; C. A. Smith, Jour. Amer. Med. Assoc., Aug. 27, 1904.

To hatch the eggs artificially Smith recommends to mix the fecal materal with a small amount of soil in a Petri dish, using a sufficient amount of water for the purpose. There should be just sufficient moisture to keep the soil damp. If there is too much the cover is left off for an hour or so. Every two to three days a few drops of water are added to replenish the moisture. Under favorable conditions in this respect all the eggs will hatch within twenty-four hours; otherwise several days will elapse. In such cultures the larvæ will remain alive for three or four months and can be observed with a ⅔ in the inverted dish.

Trichocephalus hominis, Schwank: *syn.*, Trichocephalus dispar (Rudolphi); mastigodes (Zeder); trichuris (Büttner). This parasite, which belongs to the family *Trichotrachelides,* is formed like a whip, the last end being the head end, while the tail end is very much thicker. The male measures 46 mm. and the female 50 mm. in length. The eggs are brownish in color, measuring 0.05 by 0.06 mm. in size, and present a doubly contoured shell, with a depression at each end, closed by a lid. The contents are coarsely granular. The organism is said to be the most widely distributed intestinal parasite, occurring in Europe, North America, Asia, Africa, and Australia. Its habitat is the cecum. The living worm is only rarely found in the feces[1] (Fig. 104).

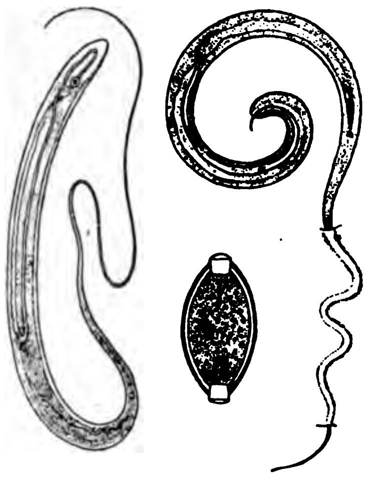

FIG. 104.—Trichocephalus trichiuris. On the left, male; on the right, female with the anterior extremity embedded in the mucous membrane of the intestine. Below, egg.

Trichina spiralis, Owen, is rarely found in the feces. The male measures 1.5 mm. in length, and is provided with four papillæ between the conical lips. The female is 3 mm. long. The uterus is situated nearer the head than the ovary, which opens into it. Fertilization occurs in the intestinal canal. The eggs develop into embryos in the uterus, emerge from this, and penetrate the intestinal walls, whence they are carried by the blood current to the muscles. The diagnosis of sporadic cases has been greatly facilitated by the discovery of Brown that eosinophilia, often of high grade, is practically of constant occurrence during the acute stage of the disease. In doubtful cases a small piece of muscle tissue (biceps, gastrocnemius) may be excised and examined for young trichinas. With the naked eye the cysts appear as minute little white specks. The worms can be rendered easily visible by placing a bit of the tissue in glycerin containing 5 per cent. of acetic acid; after a few minutes it is pressed out between two slides and examined with a low power (Fig. 105). While it is believed that trichinosis is less common in the United States than in Germany, there can be no doubt that it is not nearly as rare as was believed. Many light cases go practically unrecognized.

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[1] Ermi, Berlin. klin. Woch., 1886, vol. xxiii, p. 614.

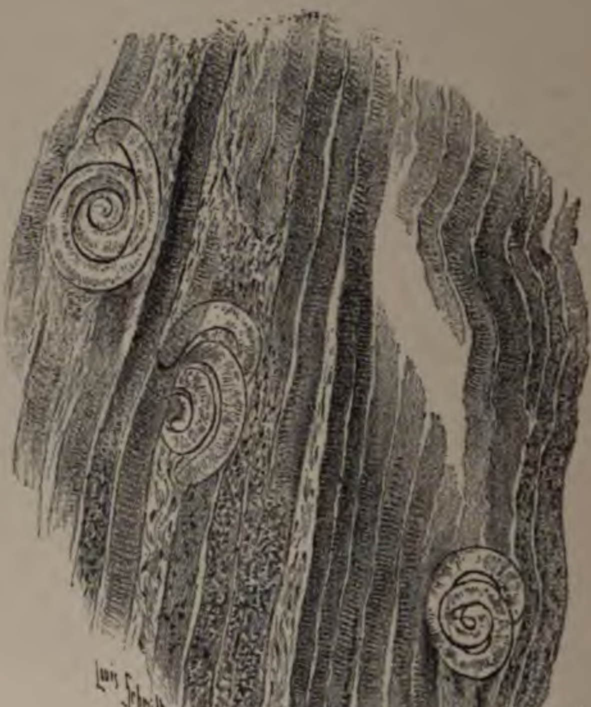


Fig. 105.—Trichina spiralis in muscle.

Strongyloides intestinalis (Bavay): *syn.*, Anguillula intestinalis (Bavay); Anguillula stercoralis (Bavay); Rhabditis stercoralis

(Bavay); Leptodera stercoralis (Bavay, Cobbold), Leptodera intestinalis (Bavay, Cobbold); Strongyloides intestinalis (Bavay, Grassi); Pseudorhabditis stercoralis (Bavay, Perroncito); Rhabdonema, strongyloides (Leuckart); Rhabdonema intestinale (Bavay, Blanchard).

In the feces of patients infested with the parasite in question the eggs of the mother-worm are only rarely found, and the adult worm itself probably never appears unless an anthelmintic has been administered and active catharsis established. Instead we find embryos (rhabditic form) measuring about 0.33 by 0.022 mm. in size. If the stools are kept, uncovered, at a temperature of about 37° C., their larvæ undergo development and reach full growth and sexual differentiation in almost five days. The length of the full-grown female is about 1 mm.; its breadth about 0.04 mm. The body is cylindrical, slightly diminishing in size anteriorly and tapering to a sharp point posteriorly. When the worm retracts forcibly, slight transverse furrows may be seen. The mouth possesses distinct lips and is continuous with a triangular esophagus, which beyond a constriction dilates again into a second ovoid enlargement. The intestine which follows ends in a little protrusion on one side of the body near the base of the tail. A little below the middle of the body, and on the ventral side, is the vulva, which leads to the uterus, extending from the intestinal ventricle to a point near the anus. Here the eggs may be massed in varying numbers. Sometimes the young have actually broken the shell of their eggs and may be seen free in the uterus; but more commonly the ova, on deposition, contain well-formed motile embryos (filariform brood). The male is about one-fifth smaller than the female. The testicle ends at the base of the tail, in two small, horn-like spicules with tapering ends, which are curved inward. These spicules contain canals; they are of equal size and situated symmetrically on a transverse plan. The tail is coiled in the same direction as the spicules, and is half as long as that of the female.

The sexually mature and differentiated forms just described represent the *Anguillula stercoralis* of Bavay. They represent an intermediate generation, developing outside of the body, which forms a link in the chain of development of the mother-worm, the *Anguillula intestinalis* (Leuckart).

Ordinarily infection takes place through the larvæ of the sexually differentiated form. These filariform embryos are longer than the rhabditiform brood of Anguillula intestinalis (Fig. 106). They are provided with a cylindrical esophagus descending down to about the middle of the body, and a tail, which, instead of terminating in a fine point, is apparently truncated at its extremity. On maturation they give rise to the Anguillula intestinalis, which is encountered throughout the upper gastro-intestinal tract, especially in the lower part of the duodenum and the upper part of the jejunum, though occasionally they have also been found throughout the entire jejunum and in the upper part of the ileum. On several occasions they have been found in the stomach.

Anguillula intestinalis, viz., the parasitic mother-worm, is, according to Rovelli, parthenogenetic, while Leuckart expressed the opinion that it might be hermaphroditic. Its length is about 2.20 mm., and its average breadth 0.03 mm. The body tapers a little anteriorly, and terminates posteriorly in a conical tail, the extremity of which is appreciably rounded and even a trifle dilated. The mouth

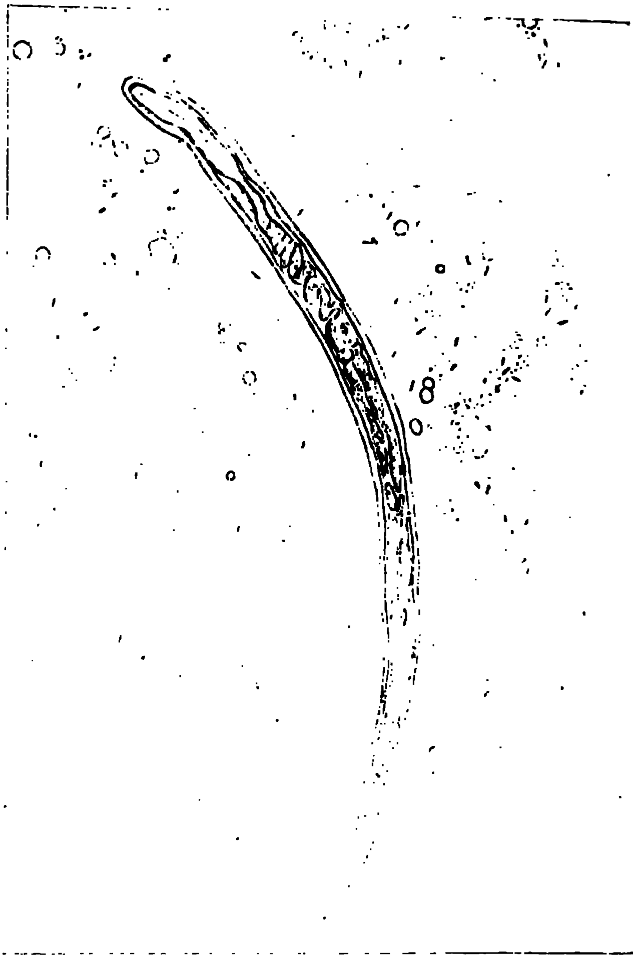
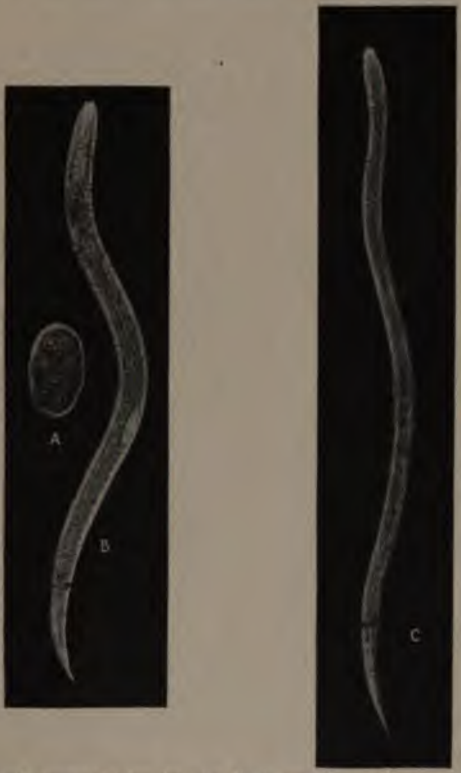


FIG. 106.—Strongyloides embryo (rhabditiform variety). The stool contained many red cells.

thout horny armature, and shows three small lips. It opens a cylindrical esophagus, which occupies about one-fourth of ngth of the animal, and shows neither swellings nor striations. intestine extends nearly to the posterior extremity of the body, s almost invisible in the middle part owing to the presence of ge, elongated ovary. The vulva is situated in the posterior of the animal, and the uterus contains usually five or six rather ated ova. The anus is situated toward the base of the tail.



107.—*A*, egg of Strongyloides intestinalis (parasitic mother-worm); *B*, rhabditiform ; *C*, filariform embryo, derived by direct transformation from a rhabditiform . (Taken from Thayer.)

eggs are of a yellowish-green color, rather opaque, and appar- finely granular (Bavay); in their general appearance they ble those of the uncinaria (Fig. 107).

hile infection originally takes place through the filariform larvæ nguillula stercoralis, an auto-infection with the larvæ may also without the intervention of the sexually differentiated forms, direct transformation from the rhabditiform embryos of the sitic mother-animal, and there is evidence to show that this latter cycle is indeed more common There is no evidence to show that the sexually mature intermediate generation ever develops in the intestinal tract during life.

The time elapsing between infection with the filariform larvæ and the appearance of rhabditiform embryos in the stools is about seventeen days.

The parasite is the recognized cause of the so-called Cochin-China diarrhea, and is of further interest from its resemblance to Ankylostoma duodenale, with which it is not infrequently found associated. Excepting in very rare instances, it does not cause intestinal ulceration, and it is supposed that the injurious effects of the parasite are purely mechanical. It is possible, however, that these may also be owing to the irritating action of its excretory products. The clinical manifestations of the disease are mainly those of a chronic diarrhea and a comparatively mild anemia. There are usually three or four pasty stools a day.

The organism was first discovered in individuals who had contracted severe diarrhea in Cochin-China. Grassi and Parona later found the worm in Italy, and at the building of the St. Gothard tunnel it was frequently seen in association with the ankylostoma. Thayer was the first to find it in the United States, and it is interesting to note that two of his three cases must have become infected in either Maryland or Virginia. The third case may have originated in Austria; in it the anguillula was associated with amebas and the Trichomonas intestinalis; it ended fatally, being complicated by liver abscess. Since then additional cases have been reported in the United States by Moore, Price, Lamar, and others.

Other cases have been observed in Belgium, Holland, Martinique, Brazil, Sicily, the Dutch Indies, Egypt, Germany, Spain, and the Philippine Islands.

LITERATURE.--Grassi, Centralbl. f. Bakt. u. Parasit., 1887, vol. ii, p. 413. Leichtenstern, Deutsch. med. Woch., 1898, p. 118. Perroncito, Arch. p. l. sci. méd., 1881, No. 2. Compt.-rend. de l'Acad. des sci., 1882, No. 1. Teissier, ibid., vol. cxxi, p. 171. Bavay, ibid., 1876, vol. lxxxiii, p. 694; ibid., 1877, vol. lxxxiv, p. 266. Normand, ibid., 1876, p. 316. W. S. Thayer, Jour. of Exper. Med., 1901, vol. vi, No. 1 (full literature to 1901). M. L. Price, Jour. Amer. Med. Assoc., Sept. 12, 1903 (literature to date since Thayer's paper).

Chemistry of the Feces.

Reaction.—The reaction of the feces is normally usually alkaline, sometimes neutral, rarely acid, the alkalinity being due to ammoniacal fermentation, the acidity to lactic and butyric acid fermentation.

In disease also the reaction of the stools is variable and of but little clinical interest. In typhoid fever an alkaline reaction is so constantly met with that this symptom might possibly be of value in doubtful cases. It may, however, also be neutral, amphoteric, or even acid. In acute infantile diarrhea an acid reaction is the rule, but exceptions also are not infrequent. Normal stools of sucklings are acid, the degree of acidity, according to Langstein, corresponding to about 2.1 to 3.7 per cent. of normal NaOH for 100 grams of the moist feces.

General Composition.—The following table, taken from Gautier, will give an idea of the composition of fresh feces, calculated for 1000 parts by weight:

	Adult man.	Suckling.
Water	733.00	851.3
Solids	267.00	148.7
Total organic material	208.75	137.1[1]
Total mineral material	10.95[2]	13.6
Alimentary residue	83.00	

The organic material yielded:

Aqueous extract	53.40	53.50
Alcoholic extract	41.65	8.20
Ethereal extract	30.70	17.60[3]

In addition, there are gases, which vary in quantity according to the nature of the food ingested, such articles as beans, bread, potatoes, etc., increasing the amount very considerably.

	Milk diet. Per cent.	Meat diet. Per cent.	Vegetable diet. Per cent.
Carbon dioxide	9–16	8–13	21–34
Hydrogen	43–54	0.7–3	1.5–4
Marsh gas	0.09	26–37	44–55
Nitrogen	36–38	45–64	10–19

Of these gases, carbon dioxide is partly referable to alcoholic and butyric acid fermentation, and partly to albuminous putrefaction. Marsh gas is formed during the fermentation of cellulose, while the nitrogen has partly been swallowed and is partly referable to albuminous putrefaction. A portion also is probably derived from the blood, and it may be mentioned in this connection that the enormous quantities of carbon dioxide so often discharged in cases of hysteria are undoubtedly referable to this source, the gas passing from the blood through the gastro-intestinal mucous membrane into the stomach and intestines.

In order to give a general idea of the chemical constituents of the feces these may be divided into:

1. Food material which could be assimilated, such as starches, fats, and a small amount of non-assimilated albuminous material.

2. Indigestible substances, such as chlorophyll, gums, pectic products, resins, various coloring matters, nucleins, chitin, and insoluble salts; viz., silicates, sulphates, earthy phosphates, ammonio-magnesium phosphate, etc.

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[1] Including 54 parts of mucin, epithelium, and calcareous salts.
[2] Not comprising earthy phosphates.
[3] Of this 3.2 is cholesterin.

3. Products derived from the digestive canal, as mucin, transformed biliary acids, dyslysin, cholesterin, lecithin.

4. Substances in process of absorption, as emulsified fats, acids, leucin, and biliary acids.

5. Products of decomposition, referable to microbic activity, as fatty acids, comprising the entire series from acetic to acid, the latter being especially abundant; lactic acid, phenol, indol, skatol, excretin, leucin, and tyrosin; phenyl-propionic, acetic, hydroparacumaric, and parahydroxyl-phenyl-acetic ammonium carbonate, and ammonium sulphide.

6. Products of metabolism eliminated through the intestines, uric acid, and xanthin bases.

7. Pigments: stercobilin, hematin, hydrobilirubin, coloring derived from the blood, and, in abnormal conditions, bile

8. Water.

9. Gases, as carbon dioxide, marsh gas, hydrogen, and

It is impossible to give here a detailed description of the chemical constituents which have been mentioned. Only the important ones, and those especially interesting from a physiological and pathological standpoint, will be considered.

Phenol, Indol, and Skatol.—Phenol, indol, and skatol are formed during the process of albuminous putrefaction, and are constant constituents of the feces. A small portion is absorbed from the intestinal canal, and appears in the urine in combination with sulphuric acid and to a slight extent also with glucuronic acid. Previously, however, the indol and skatol are oxidized to indoxyl and skatoxyl, respectively (see Urine).

To demonstrate the presence of phenol, indol, and skatol in the feces, we may proceed as follows:

The feces are diluted with water, acidified with phosphoric acid, and distilled. Volatile fatty acids, together with phenol, indol, and skatol, pass over. The distillate is neutralized with sodium carbonate and again distilled. During this process phenol, indol, and skatol pass over, the fatty acids remaining behind as sodium salts. In order to separate phenol from indol and skatol, the distillate is alkalinized with potassium hydrate and again distilled. The phenol now remains behind, and may be obtained in pure form by distilling with sulphuric acid; in this final distillate its presence may be demonstrated by the following reactions:

1. With ferric chloride phenol yields an amethyst-blue color.

2. With bromine-water a crystalline precipitate of tribromophenol is obtained.

3. Treated with Millon's reagent—*i. e.*, the acid mercuric nitrate—a red color develops.

Indol and skatol pass over after treating the above mixture of the three with potassium hydrate and distilling. These two bodies n be separated from each other by taking advantage of their t degrees of solubility in water.[1]

forms small plates, melting at 52° C., which are easily soluble water, alcohol, and ether; its odor is feculent.

tions of indol: (1) When treated with nitric acid and a little a nitrite a crystalline red precipitate of the nitrate of nitroso-is obtained. (2) A small piece of pine wood moistened with oholic solution of indol acidified with hydrochloric acid is d a cherry red. (3) A small amount of an aqueous solution of is shaken with a few drops of Ehrlich's dimethyl-amino-ben-hyde solution (which see). A cherry-red color develops either at or upon the application of heat.

atol crystallizes in plates which melt at 95° C. They are soluble a more difficulty in water than indol, and emit a feculent odor.

eactions of skatol: (1) With nitric acid and sodium nitrite only milky cloudiness results. (2) Pure skatol does not color pine wood oistened with hydrochloric acid; but if a bit of the wood is satu-ted with a dilute alcoholic solution of skatol and then immersed strong hydrochloric acid, it assumes a cherry-red and later a luish-violet color. (3) With nitric acid of a specific gravity of 1.2 t gives a marked xanthoproteic reaction on boiling—*i. e.*, a yellow olor which turns to orange upon the addition of an excess of ammonia.

Whenever there is increased intestinal putrefaction the fatty acids, phenol, indol, and skatol, will, of course, be found in increased amounts.[2]

Fatty Acids.—The fatty acids which may be found in the feces are the following:

Formic acid	H.COOH	$= C\ H_2\ O_2$
Acetic acid	$CH_3.COOH$	$= C_2\ H_4\ O_2$
Propionic acid . . .	$CH_3.CH_2.COOH$	$= C_3\ H_6\ O_2$
Butyric acid	$CH_3.(CH_2)_2.COOH$	$= C_4\ H_8\ O_2$
Isobutyric acid . . .	$(CH_3)_2.CH.COOH$	$= C_4\ H_8\ O_2$
Valerianic acid . . .	$CH_3.(CH_2)_3.COOH$	$= C_5\ H_{10}O_2$
Caproic acid	$CH_3.(CH_2)_4.COOH$	$= C_6\ H_{12}O_2$
Capric acid	$CH_3.(CH_2)_8.COOH$	$= C_{10}H_{20}O_2$
Palmitic acid . . .	$CH_3.(CH_2)_{14}.COOH$	$= C_{16}H_{32}O_2$
Stearic acid	$CH_3.(CH_2)_{16}.COOH$	$= C_{18}H_{36}O_2$

These acids are derived partly from fats, partly from carbohydrates, and to some extent also from proteins.

Cholesterin.—Cholesterin ($C_{26}H_{44}O$) occurs in small amounts in almost all animal fluids. It is found also in various tissues of the body, especially in the brain. Its origin and mode of formation in the various organs of the body, as well as the cause of its presence in the alimentary canal, are as yet unknown. It crystallizes in colorless, transparent plates, the margins and angles of which usually present a ragged appearance. (See Fig. 75, page 272.) It is practically insoluble in water, dilute acids, and alkalies. In boiling alcohol it is readily soluble and crystallizes out from this solution on cooling; it is likewise easily soluble in ether, chloroform, and benzol.

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[1] C. E. Simon, Physiological Chemistry, Lea Bros. & Co.

[2] Ibid

Tests for cholesterin: 1. Under the microscope add a drop of concentrated sulphuric acid to some of the crystals; they gradually disappear, the edges assuming a yellowish-red color.

2. Dissolve a few crystals in chloroform, add concentrated sulphuric acid, and shake the mixture: the chloroform assumes a blood-red to a purplish-red color, while the sulphuric acid at the same time shows marked fluorescence.

The Biliary Acids.—The biliary acids found in the feces are: glycocholic acid ($C_{26}H_{43}NO_6$), taurocholic acid ($C_{26}H_{45}NSO_7$), and cholalic acid ($C_{24}H_{40}O_5$).

The two former occur normally in the bile, and can be decomposed into cholalic acid and glycocoll, and cholalic acid and taurin, respectively; as this process of decomposition takes place ordinarily in the intestines, the third acid—*i. e.*, cholalic acid—is always found in the feces.

In order to demonstrate the biliary acids, the fatty acids, phenols, indol, and skatol are first removed by distillation with phosphoric acid. The residue is taken up with water and boiled, and the filtered liquid precipitated with lead acetate and a little ammonium hydrate. The biliary salts of lead are contained in the precipitate, from which they can be removed by washing with water and finally boiling the precipitate with alcohol. The washings are filtered and the lead salts transformed into sodium salts by treating the filtrates with sodium carbonate. After further filtration the filtrate is evaporated to dryness and the residue extracted without alcohol. Upon evaporation the salts of the acids sometimes crystallize out as such, while more often a dirty amorphous precipitate is obtained, whieh may be rendered crystalline by treating with ether. The amorphous residue, however, can be employed for making the necessary tests.

Pettenkofer's Test.—A small amount of the substance is dissolved in water and treated with two-thirds its volume of concentrated sulphuric acid, care being taken that the temperature does not exceed 60° or 70° C. While stirring, a 10 per cent. solution of cane sugar is added drop by drop. If biliary acids are present, the solution assumes a beautiful red color, which on standing turns a bluish violet. This test depends upon the action of furfurol, derived from the sulphuric acid and cane sugar, upon the biliary acids.

Pigments.—The principal pigment of normal feces is termed **stercobilin,** and was first isolated from this source by Vanlair and Masius.[1] Owing to its great similarity to hydrobilirubin, it has even been regarded as identical with it, but Garrod and Hopkins[2] have conclusively shown that whereas the urobilin of the urine and the stercobilin of the feces are identical in composition, as also in properties, they differ conspicuously from hydrobilirubin, and especially in the much smaller percentage of nitrogen which they contain, viz., 4.11, as compared with 9.22 per cent. It is derived from bilirubin, and formed in the upper regions of the large intestine more especially, as the result of bacterial activity.[1] This explains the observations that as a rule the meconium and the solid excreta of the first day or two of life contain no urobilin, and that the pigment also disappears, when for any reason the bile is prevented from entering the intestinal canal.

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[1] Centralbl. f. d. med. Wiss., 1871, vol. ix, p. 369.

[2] On Urobilin, Jour. of Physiol., 1898, vol. xxii, p. 451.

To isolate the pigment from the feces, the material is first extracted with alcohol. The alcoholic extract is evaporated to dryness, the residue is extracted with water, the aqueous solution acidified with sulphuric acid and saturated with ammonium sulphate, when on shaking with chloroform or a mixture of chloroform and ether the pigment is taken up by the organic solvent.

The free pigment is a brown, amorphous substance of a characteristic odor, and melts at a temperature below 100° C. On cooling, it forms a brittle, shellac-like material, which is said to be quite characteristic. It is soluble in ether, chloroform, water, and amyl alcohol. On treating its solutions with zinc chloride and ammonia a beautiful green fluorescence is obtained. Such solutions then show three bands of absorption, of which the one between *C* and *F* is the most characteristic. (See also Urinary Urobilin.)

Test for stercobilin: A small amount of feces is stirred up in water and a few c.c. of the resultant mixture treated with an equal amount of a saturated aqueous solution of bichloride of mercury. A normal stool, owing to the presence of stercobilin, then turns a pinkish red, which is the more marked the fresher the material. A green color is abnormal and denotes the presence of bile pigment.

Bile Pigment is normally absent from the feces. It occurs in large amounts in catarrhal conditions of the small intestine, and may be demonstrated by Gmelin's method, viz., a drop of the filtered liquid, or a particle of the colored fecal matter, is brought into contact with a drop of fuming nitric acid, when the yellow color will be seen to pass through the various shades of the spectrum, the green shade being the most characteristic. At times, however, it is not possible to obtain a positive reaction in this manner, although bile pigment is present. In such cases the examination should be conducted under the microscope, and attention directed to bile-stained epithelial cells, leukocytes, particles of mucus, and crystals.

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[1] A. Schmidt, Verhandl. d. XIII. Congresses f. inn. Med., 1895, p. 320. Vaughan Harley, Brit. Med. Jour., 1896, vol. ii, p. 898. Macfadyen, Nencki, and Sieber, Arch. f. exper. Path. u. Pharmakol., 1891, vol. xxviii, p. 311.

Hematoporphyrin, to judge from the investigations of Stokvis[1] and Garrod,[2] is likewise a normal component of the feces, but occurs only in traces. Garrod states that with Saillet's[3] method, the basis of which is extraction with acetic ether, after the addition of acetic acid, he invariably found traces, comparable with those which normally are present in the urine. He also states that he found considerably larger amounts of the pigment in the meconium, both in that expelled during the first day or two of life, and in that removed from the intestines of stillborn infants.

The presence of these normal traces has been referred by some to the ingested blood-coloring matter of red meat and vegetable chlorophyll. Garrod, however, finds that the hematoporphyrin does not disappear when these articles of diet are withdrawn, and while admitting that the ingested hemoglobin and chlorophyll may possibly be converted, in part at least, into hematoporphyrin, he concludes that the greater portion is derived from endogenic sources. On the whole, the evidence seems now in favor of the view that the hematoporphyrin which is found both in the urine and in the feces originates within the liver, and is eliminated into the intestinal canal in the bile. (See also Hematoporphyrinuria.)

Purin Bodies.—The purin bases of the feces are derived from the nuclei of desquamated epithelial cells, from the nucleoproteids of bacteria and leukocytes, from the secretions of the intestinal glands and the pancreas, and from the ingested food. The normal quantity according to Schittenhelm[4] varies between 0.1109 and 0.1669 purin nitrogen. When excessive amounts of meat, thymus gland, or guanin are added to the diet a large proportion of the purin nitrogen is eliminated in the feces in the next twenty-four hours. In diarrhea the fecal purins are increased.

Guanin, adenin, xanthin, and hypoxanthin are all represented, the first two prevailing.

Mucin.—According to Hoppe-Seyler, mucin is a constant constituent of the feces, both under physiological and pathological conditions. Normally, however, it is never possible to recognize its presence either with the naked eye or with the microscope. A satisfying test for the rapid demonstration of mucin in the feces does not exist. The old test of Hoppe-Seyler indicates nucleo-albumin, but not true mucin. To this end the feces are digested with water and treated with an equal volume of milk of lime; the mixture is allowed to stand for several hours, when it is filtered and the filtrate tested with acetic acid. In the presence of nucleo-albumin a cloud develops upon addition of the acid.

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[1] Nederl. Natuur-en Geneeskundig Congres, 1899, p. 378.

[2] "The Urinary Pigments in their Pathological Aspects," Lancet, Nov. 10, 1900.

[3] Rev. de méd., 1896, vol. xvi, p. 542.

[4] Zeit. f. physiol. Chem. 1903, vol. xxxix, p. 199. Walker Hall, Jour. Pathol. and Bact., March, 1904, p. 246.

Albumin is demonstrated in the feces by treating repeatedly with water slightly acidified with acetic acid. The filtrate is then examined for albumin according to methods given elsewhere (see Urine). Under normal conditions these reactions prove negative. Pathologically, serum albumin has been observed in cases of typhoid fever and chlorosis.

Albumoses are normally absent from the feces. They have been observed in typhoid fever, dysentery, tuberculous ulceration, purulent peritonitis with perforation into the gut, atrophic cirrhosis, and carcinoma of the liver. Acholic stools are also usually rich in peptones.

The albumoses are demonstrated in the following manner: the feces are digested with water, so as to form a thin mush; they are then boiled, filtered while hot, and the filtrate examined for albumin, so as to be sure that all of this has been removed. The mucin is removed by treating with lead acetate, when the filtrate is examined for albumoses as described in the chapter on Gastric Contents.

Carbohydrates.—Of the carbohydrates, starch, glucose, and certain gums may be found. In order to demonstrate these the feces are boiled with water, filtered, and evaporated to a small volume. This solution may now be tested with phenylhydrazin or Trommer's reagent for glucose (see Urine), and with a solution of iodopotassic iodide for starch (see Saliva).

In normal breast-fed infants sugar is only demonstrable in traces in the stools. Langstein[1] finds that the presence of more than traces of glucose in the stools of milk-fed infants may be regarded as a diagnostic symptom of a catarrhal process in the duodenum.

Ptomains.—Of ptomains, only two have been isolated from the feces, viz., putrescin and cadaverin. They have been found in Asiatic cholera, in cholerina, dysentery, and in connection with cystinuria. In cholera and cystinuria their amount may be quite large. Baumann and v. Udranszky obtained 0.5 gram of the benzoylated compounds from the collected feces of twenty-four hours. Such findings are exceptional, however; more often the result is negative or traces only are found; such has been my own experience and that of others. (See Ptomains in the Urine.) In cholera the cadaverin seems to predominate, while in cystinuria more putrescin is found.

To isolate the diamins in question, the feces are digested with alcohol which has been acidified with sulphuric acid. The alcoholic extract is evaporated, the residue dissolved in water, and further benzoylated, as described in the section on Urine.

MECONIUM.

By meconium are meant those masses which are first excreted from the bowel after birth. It is a thick, tenacious, greenish-brown material which has accumulated during the intra-uterine life of the infant. Microscopically, a few cylindrical epithelial cells, a few fat droplets, numerous cholesterin crystals, bilirubin crystals, and lanugo hairs are found.

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[1] Jahresb. f. Kinderheilk., vol. vi, Heft 3.

Microörganisms are absent, but soon after suckling has commenced they appear in abundance. The most important of those which are then constantly present are the Bacillus lactis aërogenes, which predominates in the small intestine, and the Bacillus coli communis, which is found more particularly in the large intestine. Both have already been described. In addition to these, the Proteus vulgaris, Streptococcus coli brevis, Micrococcus ovalis, tetragencoccus, Saccharomyces cerevisiæ, Saccharomyces rubra, and a few less important microörganisms have been found.

Chemically, meconium contains bilirubin in considerable amount (recognizable by Gmelin's reaction), biliary acids, fatty acids, chlorides, sulphates, phosphates of the alkalies, and their earths. It does not contain urobilin, glycogen, albumoses, lactic acid, tyrosin, or leucin.

An idea may be formed of its composition from the following analysis of Zweifel:[1]

Water	79.8–80.5	per cent.
Solids	19.5–20.2	"
Mineral matter	0.978	"
Cholesterin	0.797	"
Fats	0.772	"

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[1] Hellström, Arch. f. Gynäk., 1901, vol. lxiii, Heft 3.

CHAPTER V.

THE NASAL SECRETION.

In the nasal secretion, which normally is small in amount, transparent, colorless, odorless, tenacious, and of a slightly saline taste, pavement-epithelial cells in large numbers, ciliated epithelial cells, as well as some leukocytes and an enormous number of microörganisms, are found. Its reaction is alkaline.

In acute coryza the amount is diminished at first, but soon a very copious secretion occurs, which contains numerous epithelial cells and microörganisms. When complicated with an ulcerative condition pus is observed in considerable amount.

Occasionally, as in cases of traumatism, cerebral tumors, etc., cerebrospinal fluid is discharged through the nose, and may be recognized by the fact that it is free from albumin and contains a substance which reduces Fehling's solution.

Of pathogenic organisms, the tubercle bacillus and the bacillus of glanders may occur in ulcerative diseases of the nose, their presence indicating the existence of the corresponding affection. In ozena a large diplococcus has been described by Löwenberg, which is said to be characteristic of the disease. Oidium albicans has been observed in rare cases. The Meningococcus intracellularis of Weichselbaum, which is now regarded as the cause of epidemic cerebrospinal meningitis, has also been demonstrated in the nasal secretion of healthy individuals. In ordinary cases of coryza the Micrococcus catarrhalis is frequently found.

Ascarides and other entozoa have also been found.

Charcot-Leyden crystals have been observed in the nasal secretion in cases of bronchial asthma and in connection with nasal polpyi. Their presence is usually accompanied by the simultaneous occurrence of large numbers of eosinophilic leukocytes.

LITERATURE.—Reimann, Baumgarten's Jahresber., 1888, vol. iii, p. 417, Löwenberg, Deutsch. med. Woch., 1885, vol. xi, p. 6, and 1886, vol. xii, p. 446. Tost, ibid., p. 161. Gerber u. Podack, Deutsch. Arch. f. klin. Med., 1895, vol. liv, p. 262. Leyden, Deutsch. med. Woch., 1891, vol. xvii, p. 1085. Sticker, Zeit. f. klin. Med., 1888, vol, xiv, p. 81. Nothnagel, Wien. med. Blätter, 1888, Nos. 6, 7, 8.

CHAPTER VI.

THE SPUTUM.

GENERAL TECHNIQUE.

THE sputum should be collected in receptacles so constructed as to permit of their complete and easy disinfection. The paper spit-cups which are figured in the accompanying illustrations (Figs. 108 and 109) are admirably adapted for this purpose, as they may be destroyed immediately after use.

When working with sputa which are known or suspected to be of tuberculous origin, the greatest care should be exercised to keep the expectoration from drying and becoming disseminated in the air. Negligence in this respect may result in the most serious consequences.

The macroscopic examination of sputa is most conveniently carried out by placing small portions of the material upon a plate of ordinary window-glass, of suitable size, which has been painted black upon its lower surface, and covering the same with a second, smaller plate. If it is desired to examine individual constituents which have been discovered in this manner, the upper plate is slid off until the particle in question is uncovered, when it may be removed to a microscopic slide and examined under a higher power.

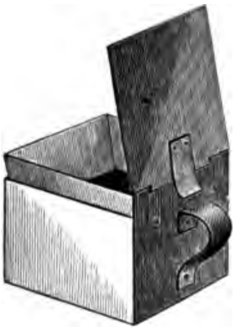
FIG. 108.

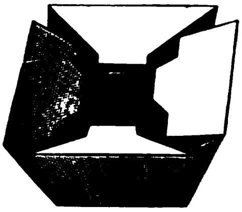
FIG. 109.

Sanitary spit-cups.

It is also very convenient to have a portion of the laboratory table painted black, when unstained plates of glass may be utilized. If these measure about 15 by 15 cm. and 10 by 10 cm., respectively, fairly large quantities of sputa may be examined *in situ* with a low power.

GENERAL CHARACTERISTICS OF SPUTA.

Amount.—The amount of sputum expectorated in the twenty-four hours varies within wide limits, depending largely upon the nature of the disease. Thus, only a few cubic centimeters may be eliminated, or the amount may reach 600 to 1000 c.c., and even more. Very large quantities are expectorated in cases of pulmonary hemorrhage and edema of the lungs, sometimes following thoracentesis, also following perforation of accumulations of pus from the thoracic or abdominal cavities into the respiratory passages; furthermore, in cases in which large vomicæ of tuberculous or gangrenous origin exist, and finally in cases of abscess of the lung, bronchiectasis, and even in simple bronchial blennorrhea. In incipient phthisis, acute bronchitis, and in the first and second stages of pneumonia, on the other hand, the amount is usually small.

In private practice, as well as in hospital work, an idea should always be formed of the amount expectorated in the twenty-four hours, especially in cases in which this is abundant. It is apparent that a copious and long-continued expectoration cannot continue without exerting very detrimental effects upon the patient's general nutrition; in cases of pulmonary phthisis, for example, Renk has shown that 3.8 per cent. of all nitrogen eliminated in such cases is removed in this manner. Lenz in his experiments found even 5 per cent.

Consistence.—The consistence of the sputum corresponds, in a general way at least, to its amount, and may vary from a liquid to a highly tenacious state. The cause of the tenacity of the sputum is but imperfectly understood. Mucin does not appear to be the most important factor, as this occurs in diminished amount in pneumonic sputa, which are noted for their high degree of tenacity. Kossel[1] has suggested that the phenomenon may be due to the presence of nucleins or nuclein derivatives, while others refer it to the presence of abnormal albuminous bodies of unknown character. However this may be, sputa are not infrequently seen where it is possible to invert the cup without losing a drop of its contents. This is observed especially in cases of acute croupous pneumonia up to the time of the crisis, providing that a catarrh of the bronchi does not exist at the same time. It is noted, furthermore, immediately after an attack of acute bronchial asthma, and also in the intial stage of acute bronchitis. In cases of edema of the lungs, on the other hand, the sputa are liquid and present the general characteristics of blood serum, being covered, like all albuminous liquids when brought into contact with the air, by a frothy surface layer. The sputa observed in cases of acute pulmonary gangrene, pulmonary abscess, putrid bronchitis, and following perforation into the lungs of an empyema or an accumulation of pus situated beneath the diaphragm, are fluid and consist of pure pus.

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[1] Zeit. f. klin. Med, 1888, vol. xiii, p. 152.

Color.—The color of the sputa may vary greatly. They may be perfectly clear and transparent, gray, yellow, green, red, brown, and even black. Purely mucoid expectoration is almost transparent and colorless, as is also the sputum of pulmonary edema when not mixed with blood or pus.

The larger the number of leukocytes the more opaque does the sputum become, assuming at first a white, then a yellow, and finally a greenish color, the latter being usually indicative of the presence of pus. The green color, however, may be due to other causes. Green sputa may thus be observed when bile pigment has become admixed with the sputa, as in cases of liver abscess perforating into the lung, or in cases of jaundice, and especially in pneumonia during lysis, in pneumonia ending in abscess, and in subacute, caseous pneumonia. The same is seen in pulmonary chloroma and may also occur in pulmonary carcinoma. In cases of amebic liver abscess with perforation into the lung the sputa usually present a color resembling anchovy sauce, which is very characteristic.[1]

The inhalation of particles of carbon gives the sputum a grayish or even a black color; the same or an ochre-yellow or red color is observed in cases of siderosis due to oxide of iron. Blue sputa are seen in workers with blue dyes (methylene blue, ultramarine), etc.

A red color is usually indicative of the presence of *blood*, the shade depending upon the character of the disease. It is seen especially after the formation of cavities, in caseous pneumonia, in incipient phthisis, heart disease, etc. The shade will further depend upon the length of time that the blood, no matter what its origin may be, has remained in the lungs. In pulmonary gangrene a dirty, brownish-red color is observed, owing to the presence of methemoglobin, and, to some extent also, of hematin. Quite characteristic is the chocolate color which is observed when a croupous pneumonia terminates in necrosis and gangrene. Equally characteristic is the rusty and prune-colored expectoration seen in ordinary cases of pneumonia. Occasionally a breadcrust brown is observed in cases of gangrene and abscess of the lung, the color being due to the presence of hematoidin or bilirubin. A light-brown color may be seen in cases of chronic passive congestion, as in mitral disease.

Odor.—Most sputa are odorless. Under certain conditions, however, there may be a marked odor. In cases of pulmonary gangrene or putrid bronchitis the stench is frightful. A somewhat similar, slightly sweetish odor is observed in certain cases in which putrefactive organisms have entered the lungs, and there exert their action upon the accumulated sputa, in the absence of gangrene, as in cases of bronchiectasis, perforating empyema, and where ulcerative processes are taking place in the lungs, whether these be of tuberculous origin or not. An odor like that of old cheese is occasionally observed in cases of perforating empyema; under such conditions tyrosin is usually found. This body, however, has nothing to do with the odor of the sputa; both factors are merely indicative of certain putrefactive changes going on in the lungs.

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[1] See C. E. Simon, Johns Hopkins Hosp. Bull., November, 1890.

Specific Gravity.—The specific gravity of sputa varies within wide limits; mucous sputa have a specific gravity of 1.004 to 1.008, purulent sputa one of 1.015 to 1.026, and serous sputa one of 1.037 or more.

Configuration of Sputa.—As a general rule, the following forms of sputa, which may be termed pure sputa, present a homogeneous appearance:

Mucoid sputa,	Homogeneous sputa,
Purulent sputa,	
Serous sputa,	
Sanguinous sputa,	

with one exception, perhaps—the typically rusty sputa of croupous pneumonia; while mixtures of any two or three of these may be classed as heterogeneous sputa:

Mucopurulent sputa,	Heterogeneous sputa.
Mucoserous sputa,	
Serosanguinous sputa,	
Sanguino-mucopurulent sputa	

The so-called *sputum crudum* of the first stage of acute bronchitis may be regarded as an example of a purely mucoid sputum. A purely purulent sputum is usually indicative of the perforation of an empyema or any other accumulation of pus into the lungs or bronchi, of pulmonary abscess, or of bronchial blennorrhea. A purely serous sputum is found in cases of pulmonary edema, and a purely hemorrhagic sputum in cases of pulmonary hemorrhage.

Of the heterogeneous sputa, the most important are the so-called *nummular sputa* of the second and third stages of phthisis. These are characterized by the fact that when thrown or expectorated into water they sink to the bottom, and there form coin-like disks, from which property they have received there name. Such sputa are mucopurulent in character, and contain a focus of almost pure pus embedded in a more or less homogeneous mass of mucus. Quite different from these are the so-called *sputa globosa*, which consist of fairly dense, roundish, grayish-white masses; they are secreted in old cavities which have become lined with a granulation membrane.

Occasionally, as in putrid bronchitis, bronchorrhea, bronchiectasis, and gangrene of the lungs, exquisite *sedimentation* is observed. Such sputa when collected in a conical glass present three distinct zones:

the one at the bottom contains the cellular elements, the second the pus serum; the third or superficial layer consists of mucus and contains many air bubbles. From this long shreds of sedimentous material sometimes hang down.

MACROSCOPIC CONSTITUENTS OF SPUTA.

Cheesy Particles.—The presence of small, *cheesy particles*, which are occasionally found at the bottom of the spit cup is sometimes very important. They vary in size from that of a millet-seed to that of a pea, and are observed especially in the second and third stages of phthisis. Usually they contain tubercle bacilli in large numbers, and frequently also elastic tissue. Not to be confounded with these are small, caseous masses which are at times expectorated by perfectly normal individuals, and also by patients suffering from acute tonsillitis, ozena, etc., and which in part come from the tonsils or mucous cysts (Dittrich's plugs); others may be derived from the bronchi. Formerly they were regarded as tubercles, and in hypochondriac individuals their expectoration may cause a great deal of anxiety. As a rule, they are expectorated unaccompanied by pus or even mucus; rubbed between the fingers they emit an extremely offensive odor, which is referable to the presence of fatty acids; microscopically they consist of bacteria, fatty acid crystals, fat globules, and cellular detritus.

Elastic Tissue.—In cases in which active parenchymatous destruction of the lungs is going on bits of elastic tissue may be found which are visible with the naked eye. The search is facilitated by spreading out the sputum between two plates of glass, upon a dark background, and searching with a hand lens. In tuberculosis the particles are quite small, while in abscess and gangrene they may attain the size of a pea. Their macroscopic demonstration should be followed by a careful microscopic examination (which see).

Particles of cartilage from tuberculous ulcers of the larynx, trachi, and bronchi are less common, as is also the occurrence of tumor fragments.

Fibrinous Casts.—Fibrinous casts are observed in croupous pneumonia, immediately before or after resolution has taken place, as also in fibrinous bronchitis (Fig. 110), and in diphtheria when the membrane has extended into the finer ramifications of the bronchi. These casts may vary in size from 15 cm. in length by several millimeters in thickness to fragments which measure only from 0.5 to 3 cm. in length. The casts observed in pneumonia, usually from the third to the seventh day, are of the latter size or even smaller, being derived from the ultimate twigs of the finest bronchioles. Those found in fibrinous bronchitis stand between these two in size, being casts of smaller and medium-sized bronchi. Attention is usually attracted to the presence of such casts by their white color; often, however, they are yellowish brown or reddish yellow, owing to the presence of blood-coloring matter; at other times they are enveloped in mucus, when their recognition may become quite difficult. Such casts are fairly firm; they branch dichotomously, usually six to ten times. The larger branches contain a lumen, while the smallest twigs are solid. Microscopically they consist of a large number of fibers, which are arranged longitudinally or in a net-like manner, and contain blood corpuscles and epithelial cells in their meshes. When treated with Weigert's fibrin-stain, they are sometimes beautifully resolved; at other times the fibrin reaction is not nearly so marked as one would expect. The individual casts consist of a variable number of lamina arranged concentrically, those contained in the centre being much folded and involuted. Most of the branches are cylindrical; some of the larger ones are flat. Charcot-Leyden crystals have at times been observed in these formations.

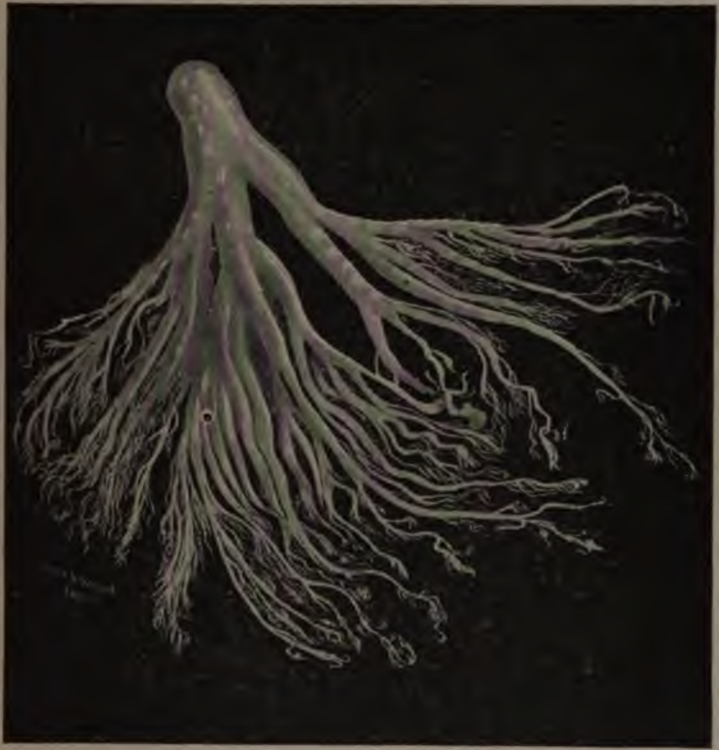

FIG. 110.—Expectorated cast from a case of fibrinous bronchitis. Three-fourths natura size. Drawn from fresh specimen. (After Bettmann.)

Small casts composed of the mycelium of fungi have also been described.

Whenever it is desired to examine sputa for casts, it is best to pick out particles that look promising, upon a dark surface, and then to shake them out in water.

Literature.—M. Bettmann, Amer. Jour. Med. Sci., 1902, vol. cxxiii, p. 304 (a full review of all cases in the literature up to 1902 is here given). Devillers and Renon, La presse médicale, 1899.

Curschmann's Spirals.[1]—Quite distinct from the formations just described are the so-called spirals of Curschmann, which are observed especially in cases of true bronchial asthma, but occur also in acute and chronic bronchitis, in croupous pneumonia and in chronic phthisis, though to a far less extent. Upon careful examination they will be seen to consist of thick, yellowish-white masses, which exhibit a spirally twisted appearance, and are characterized, moreover, by their more solid consistence and light color. Microscopically they are composed of a spirally twisted network of extremely delicate fibrils, containing epithelial cells and numerous leukocytes; the latter are almost all of the eosinophilic variety.[2] Usually, but not invariably, Charcot-Leyden crystals also are seen.[3] The spirally twisted mass is found to be wound around a central, very light and clear thread, which usually has a zigzag course (Fig. 111).

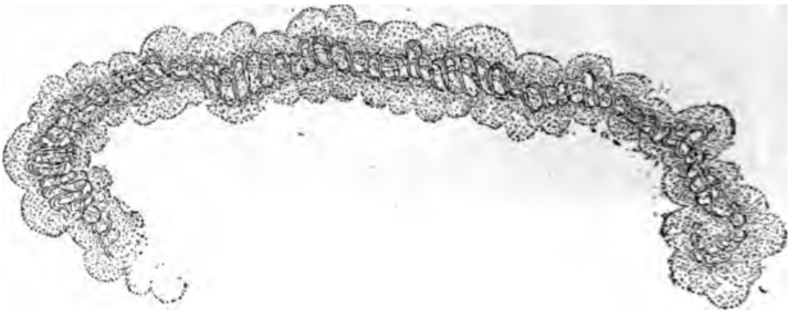

Fig. 111.—A Curschmann spiral from a case of true bronchial asthma. (Enlarged.)

Other formations, probably mere varieties of those just described, have also been observed, in which the central thread is absent or in which the spiral arrangement is deficient. The spiral form, however, with the central thread, must be considered as the most characteristic. Their length and breadth may vary a great deal, but rarely exceed 1 to 1.5 cm. Their occurrence seems always to indicate a desquamative catarrh of the bronchi and alveoli, but practically nothing is known concerning their formation. If in a given case the diagnosis rests between true bronchial and what may be termed reflex asthma, the presence of these formations points to the existence of the former disease. Chemically, the spirally wound mass seems to consist of a mucinous substance, while the central thread is possibly of fibrinous origin.

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[1] Leyden, Virchow's Archiv, 1872, vol. liv, p. 328. Curschmann, Deutsch Arch. f. klin. Med., 1883, vol. xxxii, p. 1, and vol. xxxvi, p. 578. v. Jaksch, Centralbl. f. klin. Med., 1883, vol. iv, p. 497.

[2] Schmidt, Zeit. f. klin. Med., 1892, vol. xx, p. 92. v. Noorden, ibid., p. 98.

[3] Leyden, loc. cit.

Charcot-Leyden crystals (Fig. 112), which are usually absent at the beginning of an attack of asthma, at which time only the spirals are observed, may develop from the spirals when these are kept for several days. They will be considered later in studying the chemistry of the sputum.

Echinococcus Membranes.—Echinococcus membranes may come from a perforating cyst of the liver, kidney, or lung. They constitute rather thick, and at the same time tough, pieces of membrane (Fig. 113); occasionally entire sacs are seen, of the color of white porcelain, in sections of which it is possible to make out a fibrillated structure. (See also Animal Parasites in the Sputum.)

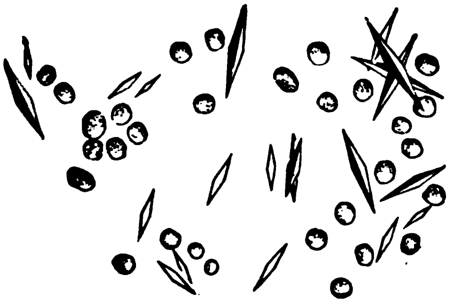

FIG. 112.—Charcot-Leyden crystals. (Scheube.)

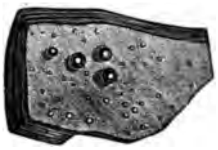

FIG. 113.—Wall of a hydatid cyst, showing the laminated structure; not magnified. (Davaine.)

Concretions.—The expectoration of concretions which have been formed in dilated portions of the bronchi or in tuberculous cavities, or of calcified bronchial glands that have found their way into the lungs is rare. Curious examples of the occurrence of such concretions have been reported. Andral cites a cases of phthisis in which within eight months 200 stones were expectorated, and Portal mentions a case in which 500 were thus expelled.[1]

Foreign Bodies.—Foreign bodies which have accidentally entered the air passages and have remained there for a long time may also be found in the sputum. Heyfelder mentions a case in which a man coughed up a wooden cigar-holder with pus and blood after eleven and a half years.

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[1] L. W. Atlee, "Bronchial Concretions," Amer. Jour. Med. Sci., 1901, vol. cxxii, p. 49. Fiessinger, "Calcule pulmonaire," Jour. de méd., 1902, No. 29.

MICROSCOPIC EXAMINATION OF THE SPUTUM.

Under this heading it is necessary to consider leukocytes, red blood corpuscles, epithelial cells, elastic fibers, corpora amylacea, parasites, and crystals.

Leukocytes.—Leukocytes, usually polynuclear in character, are found in every sputum in considerable numbers, embedded in a homogeneous, more or less tenacious material. At times they contain fat droplets, or granules of pigment, such as carbon or hematoidin. Their number varies considerably, being naturally greatest in cases of perforating abscess, empyema, putrid bronchitis, etc.

While the leukocytes which usually are found in the sputum are of the neutrophilic variety, eosinophiles may also be observed, and especially in asthmatic sputa, in which they predominate. Free eosinophilic granules are then also seen, and I have repeatedly observed specimens in which the spirals (see above) were literally covered with these granules (Plate XVII). The presence of eosinophilic leukocytes is, however, not characteristic of the sputa of bronchial asthma, as they may be met with in other diseases as well. Teichmüller has pointed out that they are present in a large percentage of tuberculous cases, and may be found months before tubercle bacilli can be demonstrated. He regards their occurrence as evidence of a defensive struggle on the part of the body, which is most evident in fairly strong individuals. In recovery a gradual increase in their number is noticeable, and a diminution, Teichmüller thinks, is indicative of a relapse, or, if the diminution occurs rapidly, of florid consumption. These statements, however, lack confirmation and are probably too dogmatic. Ott, Fuchs, Bettmann, Turban, and Cohn, in fact, deny the prognostic significance of the eosinophilic cells in cases of phthisis; and Cohn states, as the result of an examination of 100 cases, many of which were comparatively early, that the occurrence of eosinophilic leukocytes is fairly uncommon in tuberculous sputa. Stadelmann[1] also states that he has been unable to verify Teichmüller's observations. On the other hand, he has been able to confirm the observation which has been repeatedly made, that large numbers of eosinophilic cells appear in the sputum following hemoptysis. Teichmüller has also described an "eosinophilic" bronchitis, which is said to differ from other forms of the disease in the abundance of eosinophilic cells which are encountered. The sputum in such cases is described as transparent, mucoid, and loose, with yellow, purulent admixtures. It is said to be markedly different from the tough, thick sputa of bronchial asthma.

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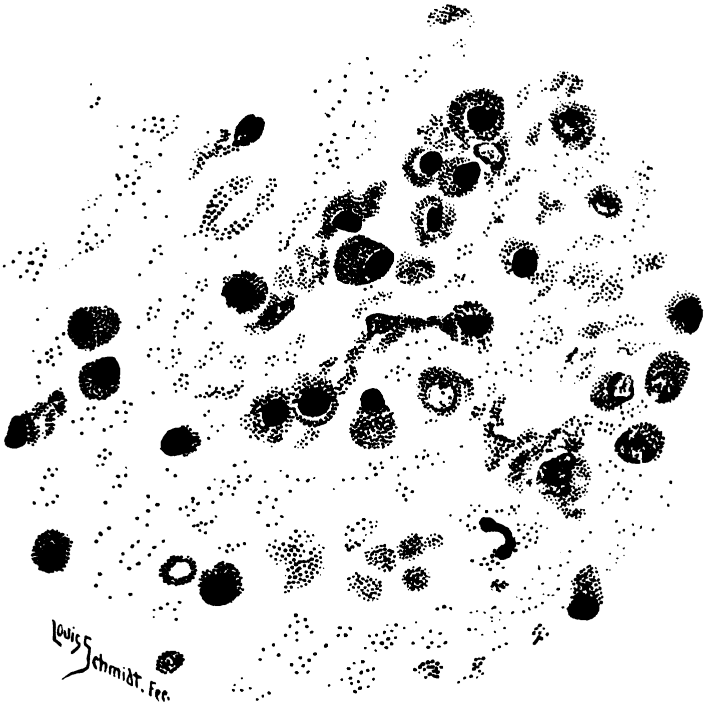
[1] Discussion on tuberculosis, Deutsch. med. Woch., 1901, vol. v, p. 210.

PLATE XVI.



Sputum from Case of Bronchial Asthma, showing Large Numbers of Eosinophilic Leukocytes and Free Granules.

It will be noted that the leukocytes are all mononuclear. (Eye-piece 1, objective 1-8, Bausch & Lomb.)

Typical spirals are absent, but rudimentary forms may be encountered. Charcot-Leyden crystals are present.[1]

Very curiously the majority of the eosinophilic cells which are met with in the sputum (notably in asthma) are mononuclear; they are not myelocytes, however, but probably mononuclear histogenetic forms.

Grünwald[2] states that in the sputa of the most diverse diseases cells are met with which contain a hypoeosinophilic granulation, and that the granules in question may also occur outside of the cells in the absence of evidence of special cell destruction. These granules, in contradistinction to the true eosinophilic cells, lose their color on treating with an acid, and readily take up the blue stain on subsequent staining with methylene blue. Grünwald states, however, that a sharp line of distinction does not exist between the two varieties of granules, and that intermediary conditions exist, as also transitions between oxyphilic and basophilic granules in the nature of an amphophilic granulation.

To demonstrate eosinophilic leukocytes in the sputum, smears are made as usual, slightly fixed by drawing through the flame of a burner, and stained for two minutes in a 0.5 per cent. alcoholic solution of eosin. The preparations are then immersed in 50 per cent. alcohol to the point of decolorization, when they are counterstained with methylene blue, briefly washed with water, and dried. The eosinophilic granules and the red cells in part hold the eosin dye.

Basophilic leukocytes (mast-cells) have also been observed in the sputa.

Red Blood Corpuscles.—The presence of red blood corpuscles in small numbers does not, by any means, indicate serious pulmonary or cardiac disease, as they may be found in almost any sputum, and especially in that of individuals who smoke much or live in a smoky atmosphere; they are, without doubt, derived from the catarrhally inflamed bronchial or tracheal mucosa. Whenever they occur in large numbers, however, their presence becomes important. They may be observed in acute bronchitis, pneumonia, edema of the lungs, bronchiectasis, abscess, gangrene—in fact, in all pulmonary diseases. Their occurrence is most important in phthisis, and is, in fact, one of the most constant symptoms of the disease.

The form of the red corpuscles will depend upon the length of time that they have remained in the lungs, and all gradations from the typical red corpuscle to its shadow, or even fragments, may be observed. In pneumonia the microscopic examination may at times be disappointing, the appearance of the sputum suggesting that red corpuscles in large numbers are present, while, as a matter of fact, they are almost all destroyed, the color being due to altered pigment. It may even be necessary to depend upon chemical methods to clear up the question. It should be remembered that the presence of blood pigment is not always indicated by a red color, but that it may also assume a golden-yellow or even a greenish tinge, owing to certain chemical changes which have taken place. The golden-yellow and the grass-green sputa observed in cases of pneumonia during convalescence belong to this class.

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[1] Teichmüller, "Die eosinophile Bronchitis," Deutsch. Arch. f. klin. Med., vol. lxiii, p. 444. See, also, K. Schönbrod, Ueber den gegenwärtigen Stand der Beurtheilung der eosinophilen Zellen im Blute und im Sputum, Inaug. Diss., Erlangen, 1895. A. Hein, Ueber das Vorkommen eosinophiler Zellen im Sputum, Inaug. Diss., Erlangen, 1894.

[2] "Studien über d. Zellen im Auswurf, etc.," Virchow's Archiv, 1899, vol. clviii, p. 297.

To demonstrate the presence of traces of blood in the sputum, the aloin or guajac test (see Feces) may be employed, after first boiling the sputum with 20 per cent. caustic alkali solution and susequently neutralizing with acetic acid.

Epithelial Cells.—Epithelial cells are found in practically every sputum. They are mostly of the pavement variety and may be derived from the mouth, pharynx, and the upper larynx. Many of the cells are full of invading bacteria, which may lead to their entire destruction. Cylindrical epithelial cells, providing they do not come from the nose, indicate in a general way an inflammatory condition of the lower larynx, trachea, or bronchi. As a rule their form is so much altered that it is often difficult to recognize them; they may thus become polyhedral, cuboidal, or even round, and can then hardly be distinguished from leukocytes. Actively moving cilia may be found only in perfectly fresh sputa, immediately after being expectorated, but are very rarely seen.

Formerly much importance was attached to the so-called *alveolar epithelial cells* (Fig. 114) as an aid in diagnosis. Buhl thus regarded them, particularly when undergoing fatty or myelin degeneration, to be pathognomonic of pulmonary disease, and especially of that form of pneumonia which has been termed essential idiopathic desquamative pneumonia. Bizzozero,[1] however, as well as others, have shown that these cells not only occur in almost every known pulmonary disease, but that they are present also in the so-called "normal" expectoration which at times is obtained upon making a forcible expiration. They are round, oval, or polygonal cells varying in size from 20 μ to 50 μ. They may contain one, two, or three oval nuclei, which are rather small and provided with nucleoli. Usually the latter are hidden beneath numerous granules. Some of the granules are albuminous, but most of them are either pigment granules, fatty granules, or myelin granules. The *myelin granules* were first discovered by Virchow[2], and termed myelin granules on

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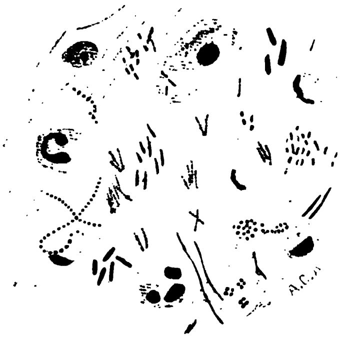
[1] Microscopie clinique. 2d ed. Française, Paris, 1885.

[2] Virchow's Archiv, 1854, vol. vi, p. 562.

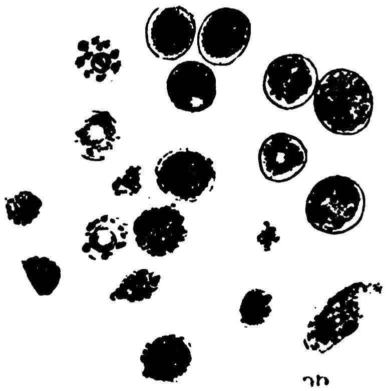
PLATE XVII.

FIG. 1.



Tuberculous Sputum Stained by Gabbett's Method. The Tubercle Bacilli are seen as Red Rods, all else is Stained Blue. (Abbott.)

FIG. 2.



Heart Disease Cells, showing Alveolar Epithelial Cells, Loaded Down with Granules of Hematin

account of their resemblance to mashed nerve matter. They are distinguished from the other forms by their clear, pale, colorless appearance, and the fact that at times fine concentric striations can be detected. These forms may be round, but more often they are irregular. Chemically, the myelin droplets have been shown to contain a considerable amount of protagon, besides traces of lecithin and cholesterin.[1] They are readily soluble in alcohol, somewhat so in chloroform and ether. They swell in water and stain yellow with iodine. They are colored but little by the anilin dyes and do not turn black on treating with osmic acid.

Sometimes myelin granules are found together with fatty and pigment granules in the same cell.

The sputa of chronic bronchitis referable to heart disease are characterized by the presence of so-called *heart-disease cells*. These are alveolar epithelial cells containing hematoidin granules (Plate XVIII, Fig. 2). They appear to be most numerous in cases of mitral disease, but may also occur in congestive affections of the bronchopulmonary apparatus, even with the heart intact.[2]

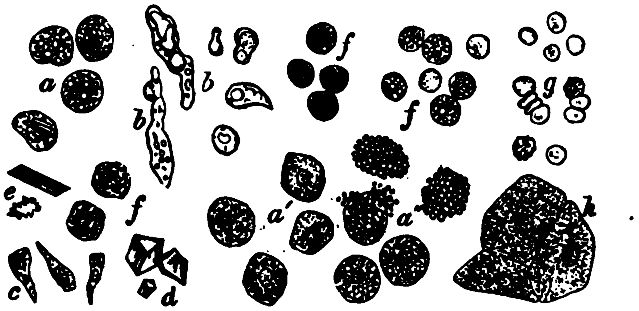


FIG. 114.—Epithelium, leukocytes, and crystals of the sputum. (Eye-piece III, objective 8 A, Reichert.) *a*, *a'*, *a''*, alveolar epithelium; *b*, myelin forms; *c*, ciliated epithelium; *d*, crystals of calcium carbonate; *e*, hematoidin crystals and masses; *f*, *f*, *f*, white blood corpuscles; *g*, red blood corpuscles; *h*, squamous epithelium. (v. Jaksch.)

Liver cells may at times be observed in the sputa in cases of liver abscess, and are easily recognized by their characteristic form.

Elastic Tissue.—Much more important from a clinical standpoint are the elastic fibers and shreds of elastic tissue which may be found in sputa. They vary much in length and breadth, and are provided with a double, undulating contour; they are usually curled at their ends. Very often they exhibit an alveolar arrangement (Fig. 115), which at once determines their origin.

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[1] A. Schmidt, "Ueber Herkunft u. chem. Natur d. Myelinformen d. Sputums," Berlin. klin. Woch., 1898, p. 73. See, also, Zoja, Maly's Jahresberichte, vol xxiv, p. 694.

[2] R. C. Regolo, Gaz. d. Ospedali, Milano, vol. xxii, No. 135.

Whenever present, elastic tissue is an absolute indication that a destructive process is going on in the lungs. It is found in cases of abscess of the lungs, bronchiectasis, occasionally in pneumonia, pulmonary gangrene and infarct, and, most important of all, in phthisis, in which it is said to be present in 90 per cent. of all cases. This percentage, which was obtained by Dettweiler and Setzer in 1878, is unquestionably too high in comparison with what is seen today, where the diagnosis of tuberculosis is made much earlier. In gangrene of the lung elastic tissue is generally said to be absent, but Osler states that he has never seen a case without it, and that usually it occurs in large fragments.

In every case it is necessary to determine whether the elastic tissue has not been ntroduced from without, and it may hence be stated as a rule that it can only be regarded as absolutely characteristic when showing the alveolar arrangement.

In order to demonstrate the presence of elastic tissue in the sputum the following method is very convenient: A small amount of the thick, purulent portion of the sputum is pressed into a thin layer between two pieces of plain window-glass, 15 by 15 cm. and 10 by 10 cm. The particles of elastic tissue appear on a black background as grayish-yellow spots, and can be examined *in situ* under a low power. Or, the upper piece of glass is slid off till the piece of tissue is uncovered, when it is picked out and examined on a slide, first with a low and then with a higher power. At first there will be some difficulty in distinguishing with the naked eye between elastic fibers and particles of bread, or milk globules, or collections of epithelium and debris, but with practice such mistakes are rarely made, and the microscope always reveals the difference.

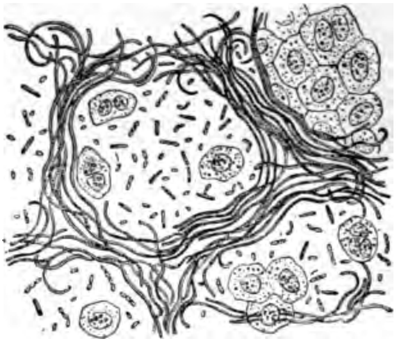

FIG. 115.—Elastic fibers in the sputum. (Eye-piece III, objective 8 A, Reichert.) (v. Jaksch.)

If only very little elastic tissue is present, it is necessary to examine large quantities of sputum with a moderately low power, and best after the addition of a solution of sodium hydrate. The sputum is boiled with a 10 per cent. solution of the reagent, an equal volume being added; the boiling is continued until a homogeneous solution has been obtained; after dilution with four times its volume of water it is allowed to settle for twenty-four hours or centrifugalized and the sediment examined at once.

May[1] recommends the following method of demonstrating the presence of elastic tissue in sputum: The material in question is heated on a boiling water bath with an equal volume of a 10 per cent. solution of sodium hydrate until it has all apparently dissolved. The mixture is then centrifugalized and the supernatant fluid decanted. The sediment is treated with about 2 c.c. of an orcein solution prepared according to the formula of Unna-Tänzer, viz., orcein, 1 gram; absolute alcohol, 80 c.c.; distilled water, 40 c.c.; concentrated hydrochloric acid, 40 drops. On adding the stain, owing to the remaining alkali, the color turns violet; a few drops (3 to 5) of hydrochloric acid are added until the original color of the stain returns. The tube is then placed for from two to five minutes in boiling water, after which acid alcohol (concentrated hydrochloric acid, 5 c.c.; 95 per cent. alcohol, 1000 c.c.; distilled water, 250 c.c.) is added to decolorize. The mixture is again centrifugalized and the sediment washed once or twice more with the acid alcohol by centrifugation and decantation. The sediment is then examined directly, when the elastic tissue fibers may be recognized by their more or less intense brownish-violet color.

ANIMAL PARASITOLOGY OF THE SPUTUM.

Protozoa. Entamœba Dysenteriæ.—In cases of amebic abscess of the liver with perforation into the lung the Amœba coli may be demonstrated in the sputa. Such sputum commonly presents the anchovy sauce appearance already mentioned. As a rule the amebas are not numerous and slide after slide may have to be examined before a single organism is discovered. The material should be kept at body temperature and the slides warmed. A Bausch and Lomb $\frac{1}{6}$ or Leitz 6 or 7 is used (see also Amebas in Feces). Only actively moving organisms are diagnostic.

Trichomonads have at times been observed in cases of gangrene of the lung, and in the pus removed postmortem from lung cavities. They are identical with the Trichomonas vaginalis of Donné.

Cercomonads have been found in the sputum and in the Dittrich plugs in gangrene of the lung.

Cestodes. Tænia Echinococcus.—Portions of echinococcus cysts, viz., pieces of membrane (Fig. 114) and hooklets (Fig. 119), are occasionally seen when the parasite has lodged in the lungs or in the neighboring organs. The disease is not common in this country. Lyon[1] collected 241 cases in the United States and Canada up to July 1, 1901. 91 per cent. occurred in foreigners. In Canada a large proportion is referable to the Icelandic immigrants in Manitoba.

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[1] Deutsch. Arch. f. klin. Med., 1900, vol. lxviii, p. 427.

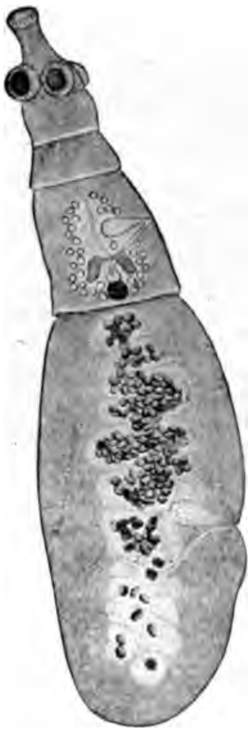

FIG. 116.

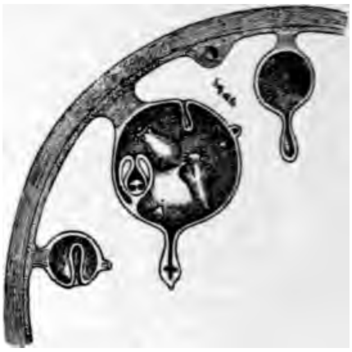

FIG. 117.

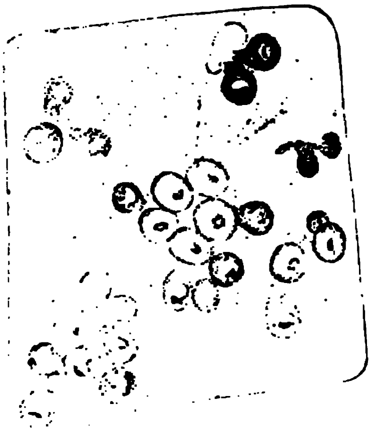

FIG. 118.

FIG. 116. Tænia echinococcus. 50. The cirrus pouch, the vagina, uterus, ovary, shell-gland and vitellogene gland and the testicular vesicles at the sides are recognizable in the second proglottis; the uterus partly filled with eggs, as well as the cirrus pouch and the vagina.

FIG. 117. Section through an echinococcus cyst with brood capsules.

FIG. 118. -A piece of the wall of an echinococcus veterinorum stretched out and seen from the internal surface. 50. A few brood capsules with scolices directed toward the interior and exterior. (Thomas.)

Thomas,[2] of Adelaide, has thoroughly investigated the disease in Australia, where it is quite common.

The adult parasite (Fig. 116), Tænia echinococcus (v. Siebold), is a three- or four-segmented tapeworm, 4 to 5 mm. in length, whose habitat is the intestinal canal of the dog, dingo, jackal, wolf, etc. The larval or cystic form develops in cattle, sheep, swine, rabbits, etc., and is also found in man. The ova, 0.067 mm. in diameter, are introduced by food, water, or by inhalation in dust. In the digestive tract the minute embryo, freed of its resistant envelope by the digesting juices of the stomach, bores its way through the intestinal wall, and finds a resting place in the liver, lung, or other part of the body, there developing into the cystic form that may attain enormous size.

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[1] N. Y. State Jour. Med., Oct., 1902.

[2] Hydatid disease, 1884.

The primary or mother cyst may produce daughter cysts, these latter granddaughter cysts, and these a third generation, often in great number; so that the cavity may be filled with cysts of varying size, formed by exogenous or endogenous growth. On the other hand, the single cyst may remain sterile—acephalocyst—or may produce scolices (Fig. 117) which are attached by pedicles to the lining of the vesicles or brood capsules in which they develop. Each scolex, or echinococcus head, 0.4 to 0.25 mm. in diameter, is a round or oval body with a head capable of protrusion or retraction. There is a single or double circlet of hooklets around, and four suckers behind the rostellum. The body is partly covered with calcareous particles. These scolices may ordinarily be found in hydatid-cyst contents.

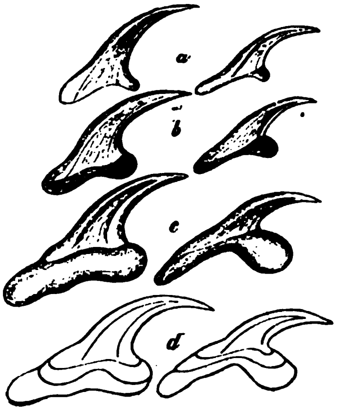

FIG. 119.—Hooklets of echinococcus: *a*, Echinococcus veterinorum: *b*, Tænia echinococcus, three weeks after infection; *c*, adult Tænia echinococcus; *d*, three forms of hooklets outlined one within the other. (Leuckart.)

Hydatid membrane (Fig. 113) varies in thickness according to the size of the cyst, a mother-cyst membrane being often ⅛ inch or thicker; the smaller cysts have walls of greater delicacy. It is usually pearly or grayish white, opaque, and of gelatinous consistency, but the thin walls of the daughter cysts may be perfectly clear and transparent. The membrane consists of two layers: (1) the ectocyst, of regular laminæ of chitinous-like material, readily torn on manipulation, the innermost layers whiter and softer than the outer; (2) the delicate, soft, granular endocyst, consisting of a mass of delicate polygonal cells without distinct nuclei. From this the scolices and daughter cysts are developed. The ectocyst usually lies in close apposition to the fibrous adventitious capsule formed by the organ in which the hydatid is present. "The ectocyst, known also as the cubicula by Continental writers, presents under the microscope a peculiar stratified structure which is quite characteristic. It shows no appearance of fibers or cells, and even under high magnifying powers it exhibits a nearly hyaline or at most a faintly granular appearance" (Thomas).

When a hydatid cyst of the lung, liver, or neighboring tissue has ruptured into the larger or smaller divisions of the bronchi, quantities of clear, watery fluid, giving the characteristic tests for hydatid fluid (see Cystic Contents), may be coughed up and be found to contain perhaps:

(*a*) Small cysts full of clear fluid, from the size of a pin's head upward—the daughter or granddaughter cysts.

(*b*) Whitish, dot-like bodies just visible to the naked eye when single, or more evident when grouped together in colonies—the scolices, or echinococcus heads (Fig. 118).

FIG. 121.

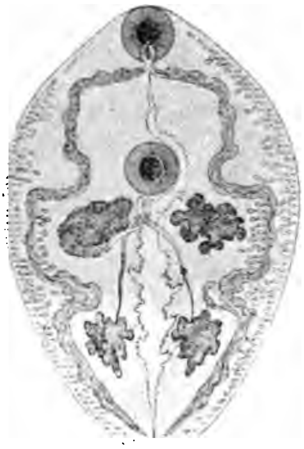

FIG. 120.

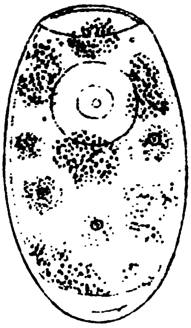

FIG. 122.

FIG. 120. Paragonimus westermanni (Kerb.). × 10. (Leuckart.) Mouth, pharynx, intestinal branches; at the sides of which the vitelline sacs are observed. The genital pore is behind the ventral sucker, and next to it, at the left, the ovary; at the right, the uterus; the two testes at the back; the excretory vessel in the middle.

FIG. 121.- Paragonimus westermanni (Kerb.) (natural size). To the left, dorsal aspect; to the right, ventral aspect. (Katsurada.)

FIG. 122 --Egg of Paragonimus westermanni (Kerb.) from the sputum. × 1000. (Katsurada.)

(*c*) Some of the component parts of the cysts or scolices, viz.:

1. Collapsed cysts—the well-known "grape skins," or pieces of the gelatinous membrane of a mother or daughter cyst.

2. Hooklets and calcareous corpuscles from the bodies of the scolices, visible only under the microscope.

Where the hydatid has suppurated before rupture, pus in large or small amount takes the place of the clear fluid or is mixed with it, the other elements being recognized on examination.

Microscopic Examination of Hydatid Material.—A piece of membrane (often yellowish and shreddy in degenerating cases) is picked up with forceps, placed on a slide, a drop or two of water applied, and lightly crushed under the cover-glass. At the torn edges of the membrane the characteristic laminated structure can be readily seen with the low power (Fig. 113). It does not stain readily, but staining is unnecessary. A section may be cut with the freezing microtome and stained with carmine.

Sputa may continue to be expectorated from a hydatid cavity of the lung for months or years, and are then usually of a purulent or mucopurulent character, perhaps blood-tinged. A thick smear on a slide may reveal, when examined with a low power, pieces of laminated membrane or hooklets. A piece of membrane, if seen on floating the sputa in water, should be picked out with forceps. Tubercle bacilli are sometimes found in the sputa of cases of pulmonary hydatid. When a hydatid of the liver has ruptured into a bronchus the sputa may be bile-stained.[1]

Trematodes. Distoma Pulmonale (Lung Fluke).—A form of pulmonary disease closely simulating phthisis and associated with pulmonary hemorrhage is very common in Japan, and has been shown to be referable to the presence of a parasite in the lungs, Distoma pulmonale (Bälz)—*syn.*, Distoma westermanni (Kerbert), Distoma Ringeri (Cobbold), Paragonimus westermanni. The parasite is 8 to 10 mm. long, 4 to 6 mm. wide, rounded very markedly in front, less so posteriorly. The color during life is a reddish brown. The two sucking disks are nearly equal in size. The ova are brown, with a thin shell and lidded. They measure from 80 to 100 μ in length and 40 to 60 μ in breadth. The worm and its ova are found in the sputum. If the sputum is shaken in water and the water renewed from time to time, in the course of a month or six weeks (according to the temperature) a ciliated embryo is developed in each ovum. When the ovum is mature, on placing it on a slide and exercising slight pressure on the cover-glass, the operculum will be forced back and the embryo will emerge and at once begin to swim and gyrate in the water (Manson). Outside of Japan the parasite has been found in Corea and Formosa. In the United States it has been found in the cat and in the dog; in the human being one case, occurring in a Japanese student, has been reported. Many Charcot-Leyden crystals are found in the sputum at the same time.

LITERATURE.—C. D. Stiles, "Distoma Westermanni," Johns Hopkins Hosp. Bull., 1894, p. 57. Brown, Die thierischen Parasiten, etc., Stuber, Würzburg, 1895.

Distoma Hæmatobium.—Manson found the ova of a species of Distoma hæmatobium in the bloody expectoration of a Chinese who had lived for some time on the island of Formosa.

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[1] For the above account of the component parts of hydatid material I am indebted to my friend Dr. John Ramsay, of Launceston, Tasmania.

BACTERIOLOGY OF THE SPUTUM.

Tubercle Bacillus.—From macroscopic examination it is impossible to decide whether or not a particular sputum is of tuberculous origin. At times a sputum may have a suspicious appearance, but it is never possible to speak with certainty from simple inspection, as a mucoid sputum may contain tubercle bacilli in large numbers, while a mucopurulent sputum may be entirely free from them, and *vice versa.* Reliance should, hence, only be placed upon a careful microscopic examination.

In all cases the fine, cheesy particles previously described should be carefully sought for, as they contain the largest number of bacilli. In their absence reliance should be placed upon the examination of a large number of preparations, attention being directed especially to the purulent and mucopurulent foci of the sputum.

If but few bacilli are present the following procedure may be employed: About 100 c.c. of sputum are boiled with double the amount of water, to which from 6 to 8 drops of a 10 per cent. solution of sodium hydrate have been added, until a homogeneous solution has been obtained, water being added from time to time to allow for evaporation. The mixture is then centrifugated or set aside for twenty-four to forty-eight hours and examined for tubercle bacilli and elastic tissue. Or, the following procedure, suggested by d'Arrigo and Stampacchia, may be employed: Four or five sputum masses are placed in a test-tube and covered with Ranvier's acid alcohol (70 per cent. alcohol, containing 1 per cent. of concentrated hydrochloric acid), so that this fills about two-thirds of the tube. The mixture is well shaken and kept, stoppered with cotton, for twenty-four hours at 37° C. or for three hours at 50° C. The acid alcohol destroys the mucus and fixes the cells and bacilli, which sink to the bottom. It is claimed that in a sediment prepared in this manner it is possible to demonstrate the tuberlce bacilli even after several years.

If, notwithstanding the fact that all due precautions have been taken, no bacilli can be demonstrated in the sputum, and the clinical history and the physical signs are indefinite or negative, the probabilities are that we are dealing with a benign process. From an examination of the sputa alone in such cases it is utterly impossible to reach a definite conclusion. When the amount of sputum, more over, is small and contains but little pus, the absence of tuberlce bacilli in doubtful cases is less suggestive of the absence of tuberculous disease than in cases in which the sputum is more abundant and mucopurulent.

Only two bacilli are likely to be mistaken for the tubercle bacillus, viz., the bacillus of leprosy and the smegma bacillus. All three are characterized by the difficulty with which they take up basic dyes, and the great tenacity with which they hold the dye when once stained, even upon treatment with mineral acids (acid fastness) and alcohol. This peculiarity has been generally referred to the presence of fat in the bacilli, but it appears from more recent researches that the chitin or chitinous substances in the bodies of the tubercle bacilli are primarily concerned in the reaction (Helbing).[1] Sata[2], moreover, has shown that other bacteria, such as the anthrax bacillus, the bacillus of glanders, the Staphylococcus aureus, etc., give a fat reaction which is as intense as that of the tubercle bacillus, while these organisms are not in the least resistant to the action of acids when stained.

That confusion should arise in the differentiation between the tubercle bacillus and the *bacillus of leprosy* is very unlikely. More important is the *smegma bacillus*, which is known to occur at times upon the tonsils, the tongue, and in the tartar of the teeth of perfectly healthy individuals. In sputum coming from the lungs it has been observed by Pappenheim,[3] Fränkel,[4] and others.

Methods of Staining the Tubercle Bacillus. 1. **Gabbett's Method.**—Bits of purulent or hemorrhagic material, or if present the cheesy particles referred to above, are spread on slides in thin layers. These are dried in the air and fixed by being passed a few times through the flame of a Bunsen burner or an alcohol lamp. The specimens are covered with a few drops of carbol-fuchsin solution[5] and heated to boiling for one-quarter to one-half minute. The solution is composed of 1 part of fuchsin dissolved in 100 parts of a 5 per cent. solution of carbolic acid and 10 parts of absolute alcohol. The excess of the staining fluid is drained off and replaced, without washing, with a solution, composed of 2 parts of methylene blue in 100 parts of a 25 per cent. solution of sulphuric acid. After a minute or two they are washed in water, dried, and examined directly in oil.

It has been suggested by Pagani[6] to use lactic acid instead of sulphuric acid, in order to avoid a too energetic decolorization. He claims that excellent results are obtained if the second solution of

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[1] "Erklärungsversuch f. d. specifische Färbbarkeit d. Tuberkelbacillen," Deutsch. med. Woch., 1900, V. B. p. 133.

[2] "Ueber d. Fettbildung durch verschiedene Bakterien," etc., Centralbl. f. allg. Path. u. path. Anat., 1900, Nos. 3, 4.

[3] "Befund v. Smegmabacillen im menschlichen Lungenauswurf," Berlin. klin. Woch., 1898, No. 37.

[4] "Einige Bemerkungen über d. Vorkommen v. Smegmabacillen im Sputum," ibid., 1898, p. 880.

[5] In its place Czaplewsky recommends the use of a solution prepared by dissolving 1 gram of fuchsin together with 5 c.c. of liquefied carbolic acid in 50 c.c. of glycerin and diluting to 100 c.c. with water. The solution does not give rise to the unsightly precipitates which are seen with the usual solution of carbol fuchsin, unless filtered.

[6] Ref. in Centralbl. f. Path. u. path. Anat., 1901, vol. xii, p. 323.

Gabbet is replaced by the following: water, 50 c.c.; alcohol, 50 c.c.; lactic acid, 2.5 grams; and methyl blue to saturation. The cover-glass specimens or slides are immersed in this solution for from fifteen to twenty seconds while gently agitating.

Gabbet's method of staining is very convenient, and is the one most generally employed. The smegma bacillus, however, is also stained.[1]

2. **The Weigert-Ehrlich Method.**—Dried specimens are prepared, and stained for twenty-four hours with a solution of fuchsin in aniline-water. The staining fluid is prepared as follows:

A test-tube full of water is shaken with about 20 drops of pure aniline oil and, after standing for a few minutes, filtered through a moistened filter. To this solution a few drops of a concentrated alcoholic solution of fuchsin or of methyl violet are added until the mixture becomes slightly cloudy—*i. e.*, until a metallic lustre is noted on the surface. After twenty-four hours the preparations are washed with water in order to remove an excess of staining fluid. They are then immersed for several seconds in a dilute solution of nitric or hydrochloric acid (1 to 6, 1 to 3, or 1 to 2), and washed again with water or with absolute alcohol. At this time the specimens should have a faintly red or violet color. They are then dried, and mounted as usual.

If it is desired to use a counter-stain, Bismarck brown, vesuvin, or methylene blue in watery solutions may be used. Into such a solution the specimen is placed after treatment with nitric acid and washing in water. It remains for about two minutes, and is then washed, dried, and mounted as above.

3. **Ziehl-Neelsen's Method.**—A mixture of 90 parts of a 5 per cent. solution of carbolic acid and 10 parts of a concentrated alcoholic solution of fuchsin is used. The procedure is the same as that described under the Weigert-Ehrlich methd. It is usually not necessary to stain the preparations for twenty-four hours, however, and as a rule it is sufficient to place a few drops of the staining fluid upon the preparation and to heat over the free flame as described when the specimen is decolorized as before. In this manner excellent results may be obtained in a few minutes.

Stained according to one of these methods, the bacilli appear as rods, measuring about 1.5 to 3.5 μ in length by 0.2 μ in breadth (Plate XVIII, Fig. 1). Much larger specimens may, however, also be seen, up to 11 μ in length. The shortest forms are commonly straight; the common types are usually slightly curved. They may occur joined in chains of two or three, and branching forms have also been observed. Occasionally one may see a couple of organisms, each bent to a crescent, linked in the form of the letter S.

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[1] Fränkel, Berlin. klin. Woch., 1884, vol. xxi, p. 195; and Deutsch. med. Woch., 1887, vol. xvii, p. 552

Very commonly they are beaded, and it is possible to make out from 1 to 8 clear spaces in an organism which are separated by round or rod-shaped granules, which are deeply stained and appear to lie in a lightly staining capsule. The small hyaline bodies were once regarded as spores, but it is more likely that they are vacuoles. Sometimes bacilli are seen which have club- or knob-shaped enlargements at the extremities. These enlargements likewise have been viewed as spores, while others look upon them as products of degeneration. When present in large numbers, the bacilli are often seen in clumps, as though they had been agglutinated, but in every specimen isolated organisms are also found scattered through the field; or two or three in groups.

Cultivation of the Tubercle Bacillus.—The cultivation of the tubercle bacillus is best accomplished on blood serum or glycerin agar (agar with 6 per cent. of glycerin added) at a temperature of 37° or 38° C. Below 30° C. and at a temperature higher than 42° C. the organism does not grow. Primary inoculation from the tissue should be made on blood serum, as the bacillus usually does not grow on glycerin agar when this is inoculated directly from the tuberculous focus. Subcultures, however, grow readily on glycerin agar and more rapidly than on blood serum. The individual colonies appear like small, dry scales, which gradually coalesce and form a wrinkled film of a dull, whitish color. Older cultures present a brownish or grayish-brown color. An adequate idea may be formed of the growth of the organism after two or three weeks. Sunlight rapidly kills the tubercle bacillus.

Number in Sputum.—The number of bacilli which may be found in a sputum varies greatly, and while in general it may be said that it is in direct ratio to the intensity of the disease, and may thus be considered of prognostic value, too much reliance should not be placed upon this statement, as in acute miliary tuberculosis, and in cases that have gone to the formation of cavities, the number may be small or they may be absent altogether. In an incipient case, on the other hand, in a little mucoid sputum the number may be large. If the number of bacilli steadily decreases in a series of examinations at intervals sufficiently long, the patient may be regarded as improving, but here the constitutional symptoms and local signs give much more accurate information.

If on repeated examination large numbers of tubercle bacilli are found, the disease has in all probability advanced to cavitation (Brown).

In tabulating the number of tubercle bacilli in reports one may adapt Gaffky's scheme, modified by L. Brown as follows ($\frac{1}{12}$ oil immersion; ocular 1; B. & L.):

1. Only 1 to 4 in a whole preparation.
2. Only 1 bacillus on an average in many fields.

3. Only 1 bacillus on an average in each field.
4. 2 to 3 bacilli on an average to each field.
5. 4 to 6 bacilli on an average to each field.
6. 7 to 12 bacilli on an average to each field.
7. 13 to 25 bacilli on an average to each field.
8. About 50 bacilli on an average to each field.
9. 100 or more bacilli on an average to each field.
10. Enormous numbers on an average to each field.

An attempt has been made to attach prognostic significance to the form and grouping of the tubercle bacilli in the sputum. To judge from the experience gathered at Saranac, it appears that virulent and attenuated forms of tubercle bacilli possess practically the same morphology and that short bacilli usually represent a younger growth. Arrangement of the bacilli in clumps is more apt to be found in the severer cases, but may occur in all (Brown).

Of the variations in number and form of the tubercle bacilli during treatment with Koch's tuberculin it is unnecessary to speak at this place, as the prognostic significance attaching to such variations is questionable.[1]

The Diplococcus Pneumoniæ.—The Diplococcus pneumoniæ of Fränkel and Weichselbaum, also commonly termed the pneumococcus, is the recognized cause of acute croupous pneumonia in the majority of cases. It is then seen in the sputum in large numbers and recognized by its capsule. It may, however, also occur in the mouth of perfectly healthy individuals, so that its diagnostic significance is somewhat limited. To demonstrate the organism smears on slides or cover-glasses are placed for one or two minutes in a 1 per cent. solution of acetic acid; they are then removed and the excess of acetic acid drawn off, when they are allowed to dry in the air; they are subsequently placed for several seconds in saturated aniline-water and gentian-violet solution, washed in water, and examined. Rod-shaped diplococci (Fig. 123), surrounded by a capsule, which latter is considered the characteristic feature of this organism, will be seen in cases of acute croupous pneumonia.[2]

As a rule the capsule is not well shown in this way. The best results are obtained with *Buerger's method.*[3] Smears are prepared as usual. As soon as the edges begin to dry they are covered with Müller's fluid,[4] saturated with bichloride of mercury (ordinarily about 5 per cent.). The specimens are gently warmed over the flame for about three seconds (using cover-glass smears), rapidly washed in water, flushed once with alcohol (80 to 95 per cent.), and then treated with ordinary tincture of iodine for one or two minutes. The iodine in turn is thoroughly washed off with alcohol and the preparations dried in the air. They are then stained for two to five seconds with gentian aniline-water (aniline-oil 10 c.c., water 100 c.c.; shake, filter, and add 5 c.c. of a saturated alcoholic solution of gentian violet; or 10 per cent. aqueous fuchsin solution, viz., saturated alcoholic solution of fuchsin 10 c.c. and water 100 c.c.). Washing with a 2 per cent. aqueous solution of salt completes the process. The preparations are examined in a drop of the salt solution and ringed with vaselin.

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[1] F. Fischel, Unters. über d. Morphol. u Biol. d. Tuberculose-Erregers, 1895. Gaffky, Mittl. aus. d. Kais. Gesundh. Anz., vol. xi, p. 126; L. Brown, Jour. Amer. Med. Assoc., 1903, vol. xl, p. 514.

[2] Fränkel, Zeit. f. klin. Med. 1886, vol. ii, p. 437. Weichselbaum, Wien. med. Woch., 1886, vol. xxxix, pp. 1301, 1339, 1367.

[3] Buerger, L. Med. News., Dec. 10, 1904.

[4] Composition of Muller's fluid: 2.5 grams potassium bichromate, 1 gram sodium sulphate, and 100 c.c. of water.

With this method there is visible a refractile, deeply staining, regularly outlined, narrow, elliptical capsule membrane, separated from the diplococcus by a clear area of capsular substance which either remains unstained or takes a faint color.

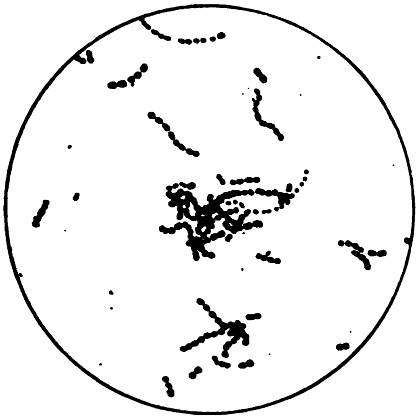

FIG. 123.--Pneumococcus from bouillon culture, resembling streptococcus. (Park.)

If smears are to be made from cultures or from material which in itself is essentially non-albuminous, Buerger directs that a drop of blood serum diluted with an equal amount of saline solution should be placed upon the slide or cover, and that the smear be made in this. Epstein finds that albumen-water (egg albumen shaken with an equal volume of water or normal salt solution) works just as well and will keep for two or three weeks.

The Bacillus of Influenza.—The bacillus of influenza was discovered in 1892 by Pfeiffer. It is found in the bronchial sputum in large numbers and is essentially characterized by its minute size, measuring only 0.2 to 0.3 μ in breadth by 0.5 μ in length (Fig. 124). The organisms occur for the most part singly, but may also form chains of threes and fours. In suitably stained specimens they may at first sight appear as diplococci, owing to the fact that the poles are stained more deeply than the intervening portion. Carbol fuchsin diluted in the proportion of 1 to 10 with water stains the bacillus very well and brings out the polar staining.

The organism is non-motile and forms no spores. It can be grown on media containing blood or serum (blood agar, hydrocele agar, Löffler's serum). Human blood and pigeon blood are the best. Growth, however, in any event is slight and occurs slowly. In order to cultivate the influenza bacillus from the sputum, this is collected in sterile cups and examined without delay. The sputa are washed in sterile bouillon or sterile normal salt solution and cultures made on blood agar. (Boggs[1] recommends pigeon-blood agar or agar to which sterile fetal blood has been added.) Tiny, water-clear colonies then develop, as described by Pfeiffer. On the fetal-blood agar Boggs noted that involution forms appear earlier and in much greater number than when pigeon, rabbit, or adult human blood was used. Some of these forms are so large and irregular as to give at first sight the impression of a mixed infection.

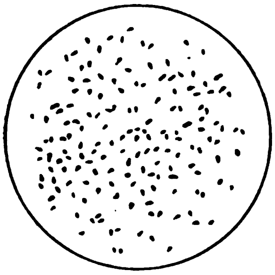

FIG. 124.—Influenza bacilli.

From the blood the organism is rarely obtained.

Influenza-like bacilli have been found in whooping-cough sputa by Spengler, Jochmann, and Krause, and more recently by Wollstein. The organism in question has been named the *Bacillus pertussis*, Eppendorf. According to Spengler the bacillus of Czaplewski and Hensel is only a contaminating pseudodiphtheria bacillus.

To cultivate the **Bacillus pertussis** the sputum masses coughed up after a paroxysm are washed in six successive beakers of peptone water and spread upon blood-agar plates prepared by mixing placental blood with melted agar. The predominating colonies are then small, transparent, dew-drop like, and not surrounded by a hemolytic zone, as in the case of the pneumococcus and streptococcus. Microscopically they appear as slightly raised, almost structureless droplets. After forty-eight hours the colonies show a slightly granular centre. The bacilli also grow in bouillon to which a drop of fresh or hemolyzed blood is added. On ascitic fluid agar, glycerin agar, Löffler's serum, plain bouillon, serum broth, milk and gelatin no growth takes place.

The organisms are not motile. They are short, plump, ovoid, with rounded ends, lying singly or in small groups between the pus and epithelial cells of the sputum They are decolorized by Gram's method. Somewhat larger forms are found in older cultures, and Spengler speaks of very long chains.

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[1] Amer. Jour., Nov., 1905, p. 902.

Wollstein[1] obtained agglutination with the serum of the corresponding child in dilutions of 1 to 200 and occasionally of 1 to 500.

The Smegma Bacillus.—In a few isolated cases the smegma bacillus has been encountered in the sputum, and, as I have already stated, the same organism may normally be present in the saliva, the coating of the tongue, the tartar of the teeth, etc. Like the tubercle bacillus, it resists the decolorizing action of acids when once stained, and may hence be confounded with it unless special precautions are observed (see Urine).

The Typhoid Bacillus.—It has been conclusively shown that the typhoid bacillus can be present in the sputum of typhoid patients, especially if there is a coexistent bronchitis or pneumonia.[2]

The Plague Bacillus.—The plague bacillus is seen in the sputum in enormous numbers in cases of the pneumonic type of the disease. By direct observation, however, it may not be recognized immediately, and it is best in every case to resort to culture as well (Fig. 44, page 176, see Blood). The organism may be found in the sputum on the first day of the disease.

Micrococcus Catarrhalis.—This organism is frequently seen in the sputa and nasal discharge. It is larger than the common staphylococci, but, like these, frequently occurs in lateral pairs, the contiguous sides being concave.

Micrococcus Tetragenus.—This organism is frequently seen in the sputum under the most varied pathological conditions and may also occur in the mouths of perfectly healthy individuals. It is a coccus occurring in fours, each measuring about 1 μ in diameter. The form which is found under normal conditions, in contradistinction to disease, cannot be cultivated.

Staphylococci and **Streptococci** may be found in the mouths of apparently healthy individuals, but are more commonly encountered in inflammatory conditions of the most divers kinds. Where cavity formation is going on in the lungs they are usually very numerous.

Streptothrices.—Within recent years there is a tendency among pathologists to abandon the older terms actinomyces, cladothrix, etc., and to speak of infections with branching mycelial organisms under the collective term streptothricosis, designating the specific variety by its special term.

Up to 1902 about 100 cases of supposed cattle *actinomycosis* had been reported in the United States, as occurring in man (Ewing), but it is difficult to say how many of the older cases really belonged to this order; in the light of recent investigations it seems not unlikely that many were referable to different species.

In the cattle disease yellow granules (so-called sulphur granules) may be found in the pus derived from actinomycotic tumors, in the sputum, and in the feces, when the disease has attacked the lungs and intestines respectively, which measure from 0.5 to 2 mm. in diameter. If such a granule is examined microscopically, slight pressure being applied to the cover-glass, it will be seen to consist of numerous threads which radiate from a centre in a fan-like manner and present club-shaped extremities (Fig. 125).

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[1] Jour. Exper. Med., 1905, vol. vii, p. 335.

[2] M. W. Richardsen, Boston Med. and Surg. Jour., Feb. 5, 1903.

The cattle organism is termed the *Streptothrix* (*Actinomyces*) *bovis communis* (Streptothrix actinomycotica, or ray fungus). It may be demonstrated in the following manner: Dried cover-glass preparations are stained for five to ten minutes with aniline-water—gentian violet (see Weigert-Ehrlich stain for tubercle bacilli), when they are rinsed in normal salt solution, dried between filter paper, and transferred for two or three minutes to a solution of iodopotassic iodide (1 to 100 or 1 to 150). They are then again dried between layers of filter paper, decolorized in xylol-aniline oil (1 to 2), washed in xylol, and mounted in balsam. The mycelium assumes a dark-blue color.[1] The organism is acid fast, but loses its color on washing with alcohol (95 per cent.).

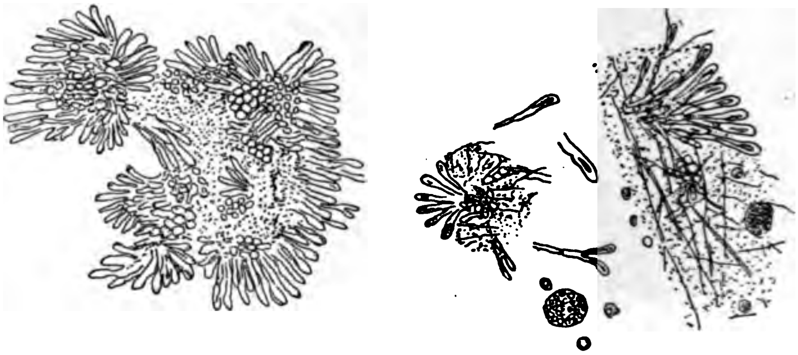

FIG. 125.- Actinomyces. (Musser.)

In addition to the cattle cases there exists a group of pulmonary cases which present the clinical features of tuberculosis, bronchopneumonia, or gangrene, but in which the infecting agent is a species of streptothrix different from the cattle variety. About 30 cases of this kind have been reported (1906). Different species have been described, such as the *Streptothrix eppingeri* (Cladothrix asteroida), Streptothrix pseudotuberculosa, Flexner; Streptothrix hominis, Foulerton, and Streptothrix israeli.

The organism is found in the sputum, often in the form of small, grayish-yellow granules. These are made up of a mycelium of branching organisms, which in the unstained specimen appear as fine, homogeneous, glistening threads, about two to four times as wide as a tubercle bacillus. They are acid fast, but can be decolorized with alcohol. In such specimens many of the threads present a beaded appearance and sometimes seem to be breaking up into short rods of varying length. With Gram some varieties stain well, while others do less so. Culture yields uncertain results. Flexner obtained no growth. Eppinger succeeded with gelatin, inspissated horse serum, maltose agar, and potato.

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[1] R. Paltauf, Sitzungsber. d. K. K. Gesellsch. d. Aerzte Wien, 1886.

LITERATURE.—Ashton and Norris, Jour. Amer. Med. Assoc. Sept. 9, 1905. Flexner, Trans. Assoc. Amer. Phys., 1898, vol. xiii. Warthin and Olney, Amer. Jour., Oct., 1904. W. G. Ewing, Johns Hopkins Hosp. Bull., 1902, vol. xiii. J. Ruhräh, Annals of Surg., 1899, vol. xxx (analysis of 62 cases).

Blastomycetes.—In the rare cases of systemic blastomycosis blastomycetes may be demonstrable in the sputum. Such a case has been described by Eisendrath and Ormsby.[1] For the examination of pus or sputum the writers recommend the addition of a little 10 per cent. NaOH solution to the specimen and to examine unstained with a $\frac{1}{6}$ or $\frac{1}{7}$ objective. The refractile parasite is thus well brought out. (Figs. 126, 127, and 128.)

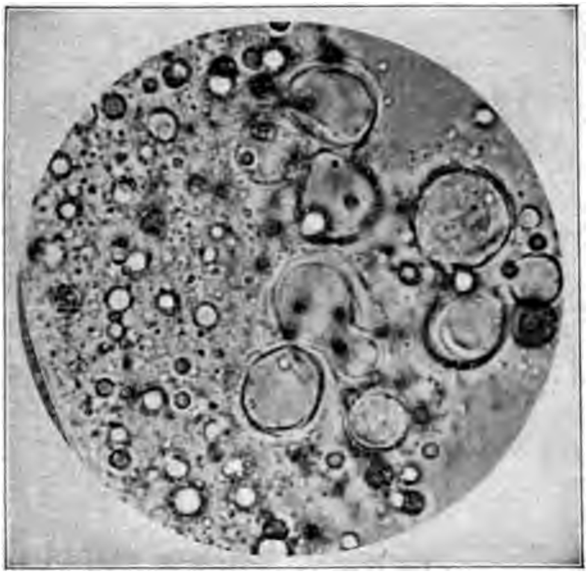

FIG. 126.—Blastomycetes. Smear from sputum mounted in 1 per cent. potassium hydrate solution, showing circular and budding organisms. × 1200. (Eisendrath and Ormsby.)

Molds.—Of other fungi which are occasionally observed, there may be mentioned various varieties of mucor and aspergillus. Some of these organisms (*Mucor corymbifer* and *Aspergillus fumigatus*) have been found associated with cavity formation and seem to have pathogenic properties. They may at times overgrow the saprophytic bacilli (*Pneumonomycosis aspergillina*, seu *mucorina*). They are best studied in the fresh specimen, not stained (Figs. 129 and 130).

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[1] Journal Medical Association, October 7, 1905.

Sarcina pulmonalis has been found at times, especially in the mycotic bronchial plugs occurring in putrid bronchitis. It is usually smaller than the Sarcina ventriculi, but larger than the variety observed in the urine; it presents the characteristic form of the latter.

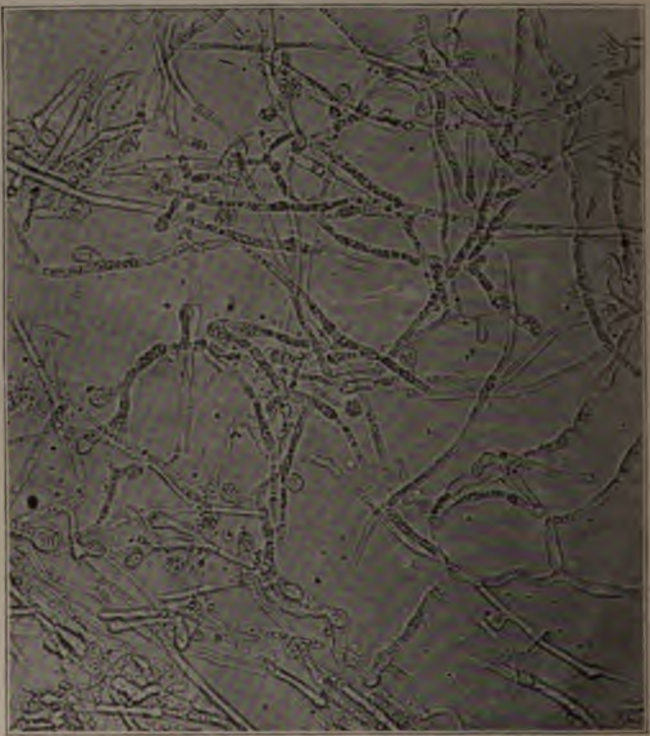

FIG. 127.—Blastomycetes. Smear from growth on media, five weeks old, in 1 per cent. potassium hydrate solution. Low power. (Eisendrath and Ormsby.)

Oidium albicans may be seen in children, and is usually derived from the mouth.

Crystals.—Of crystals which may occur in sputa, it will be necessary to consider briefly the crystals of Charcot-Leyden, hematoidin, cholesterin, margarin, tyrosin, calcium oxalate, and triple phosphates.

Charcot-Leyden Crystals.[1]—These crystals were discovered in the sputa of patients suffering from bronchial asthma, and were supposed to stand in a causative relation to the disease. This view has been abandoned, and it is known that they may occur in other diseases as well. But while their presence is almost constant in bronchial asthma at a time when Curschmann's spirals can also be demonstrated, they are only exceptionally met with in other diseases, such as acute and chronic bronchitis, phthisis, etc. They were formerly regarded as identical with *Böttcher's sperma crystals*, but it has been shown that this is not the case. They are straight, hexagonal, double pyramids, and appear under the microscope as flattened needles of variable size (Fig. 112). Some attain a length of from 40 μ to 60 μ, while others are scarcely visible even with a comparatively high power of the microscope. They show a feeble, positive, double refraction, and have but one optical axis, while the sperma crystals are biaxial and strongly double refracting. Their behavior to solvents is essentially the same as that of the sperma crystals, but they differ from these in their insolubility in formol. They are colored yellow with Florence's

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[1] Leyden, Virchow's Archiv, 1872, vol. liv, p. 324. Schreiner, Liebig's Annal., 1878, vol. cxciv, p. 68. Cohn, Centralbl. f. allg. Path. u. path. Anat., vol. x, p. 940. Brown, Phila. Med. Jour., 1898, p. 1076.

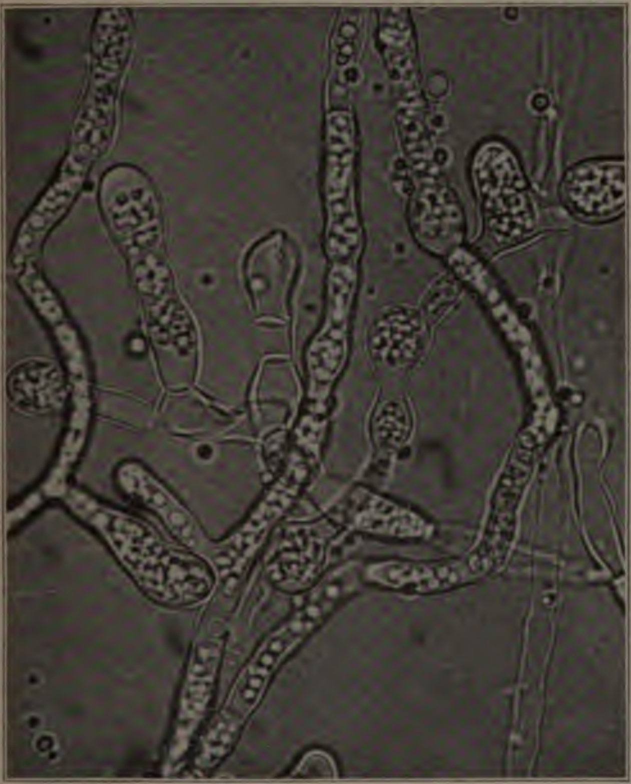

FIG. 128.—Higher magnification of Fig. 127. × 1200.

reagent, while the sperma crystals are stained a bluish black. Very curiously the appearance of Charcot-Leyden crystals is closely associated with the presence of eosinophilic leukocytes, and they have hence been termed *leukocytic crystals*. They may in fact originate within the cells. In bronchial asthma it is not uncommon to find microscopic preparations of the sputum literally studded with eosinophilic leukocytes and free granules. Outside the sputum they are also found in the blood, in myelogenous leukemia, and in the stools in association with animal parasites. They readily form in both normal and abnormal red bone-marrow, and excellent specimens may be obtained for purposes of demonstration if a piece of a rib is allowed to remain exposed to the air for a few days. The marrow then usually contains large numbers. The crystals also form in decomposing viscera in general, and at times form a complete covering of old anatomical preparations. Their occurrence may be regarded as evidence of retrogressive changes in the cellular elements of an organ. Of the relation which they bear to the eosinophilic leukocytes, with which they are so constantly associated, nothing is known. The Charcot-Leyden crystals can be stained with the triacid stain, with thionin, with the eosinate of methylene blue, and other dyes.

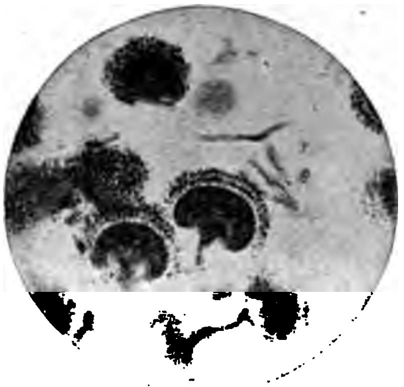

FIG. 129.—Aspergillus fumigatus. × 350. (Fränkels.)

Hematoidin crystals may be observed in the sputa following extravasations of blood into the lung. They frequently occur in the form of ruby-red columns or needles; amorphous granules, however, are also seen, enclosed in the bodies of leukocytes, in which case they are probably always indicative of a previous hemorrhage, while the needles are generally observed when an abscess or empyema has perforated into the lungs. The substance is derived from blood pigment, and is now known to be identical with bilirubin.

Cholesterin crystals are at times seen in the sputa in cases of phthisis, pulmonary abscess, and, in general, whenever old accumulations of pus have entered the lung from a neighboring organ. They are readily recognized by their characteristic form and chemical properties (see Feces).

Fatty acid crystals are frequently observed in cases of putrid bronchitis and gangrene of the lung, and also in cases of bronchiectasis and phthisis. They occur in the form of single needles or groups of needles, which are long and pointed. They are easily soluble in ether and hot alcohol; insoluble in water and acids. Chemically, they are probably composed of the higher fatty acids, such as palmitic and stearic acids.

Tyrosin crystals have been observed in cases of putrid bronchitis, perforating empyema, etc. **Leucin** is then usually also present, occurring in the form of highly refractive globules. For the recognition of these bodies, particularly of tyrosin, a chemical examination should always be made, as crystals of the soaps of fatty acids have frequently been mistaken for those of tyrosin (see Urine).

Calcium oxalate crystals are rarely seen. Fürbringer observed them in large numbers in a case of diabetes, and Unger found them in a case of asthma. They are readily recognized by their envelope form and central cross, but they occur also in amorphous masses. They are soluble in mineral acids; insoluble in water, alkalies, organic acids, alcohol, and ether.

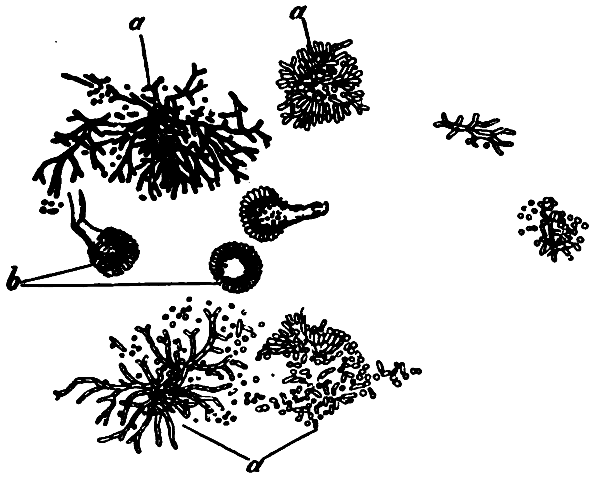

FIG. 130.—Aspergillus fumigatus of the lung, partly schematic: a, mycelium of aspergillus in roset-like rays; b, sporangium. × 285. (Weichselbaum.)

Triple phosphate crystals also are rarely seen, but may occur in cases of perforating abscesses, etc. They are recognized by their coffin-lid shape and the readiness with which they dissolve in acetic acid.

The Pneumoconioses. Anthracosis.—To some extent particles of carbon may be found in the sputum of almost every individual. The expectoration in such cases is of a pearl-gray color, and is brought up in larger or smaller masses, especially in the morning upon rising. Larger amounts are noted in miners and in those who are brought into close contact with coal-dust. Microscopically, particles of carbon and epithelial cells, of the alveolar type, as well as leukocytes loaded with the pigment, are seen.

Siderosis.—In siderosis the sputum presents a brownish-black color and contains cells enclosing particles of ferric oxide. These may be readily recognized by treating with a drop of ammonium sulphide or potassium ferrocyanide solution in the presence of hydrochloric acid, when a black color on the one hand or a blue color on the other is obtained in the presence of iron.

Chalicosis.—In chalicosis silicates are found in the sputa.[1]

CHEMISTRY OF THE SPUTUM.

In addition to the substances described, sputum contains certain albumins, volatile fatty acids, glycogen, ferments, and various inorganic salts.

Among the albumins may be mentioned serum albumin, and especially mucin, which is often present in large amounts. In pneumonic and purulent sputa albumoses also have been found.

In order to demonstrate the presence of serum albumin the sputa are treated with dilute acetic acid, when the filtrate is tested with potassium ferrocyanide, as described in the chapter on Urine. Serum albumin is, of course, found in notable quantities in cases of edema of the lungs. Especially interesting is the *albuminous expectoration* which at times follows thoracentesis. The amount of sputum usually varies between 200 and 900 grams, but may be much larger and may reach 2000 c.c. or even more. Occasionally it begins before the tapping is completed or immediately after. More commonly, however, an interval varying from five minutes to one or two hours elapses before the expectoration begins. Its duration is variable. Sometimes it lasts only a few minutes, more often an hour or two, and in rarer cases a whole day or two. The condition is probably due to edema of the lungs.[2]

The volatile fatty acids contained in sputa may be obtained by diluting with water, acidifying with phosphoric acid, and distilling, when the distillate is further examined as described in the chapter on Feces. Acetic, butyric, propionic, and capronic acids have been found.

The fats and fixed fatty acids are extracted from the residue with ether, and shaken with a solution of sodium carbonate in order to transform them into their sodium salts, when the ether is decanted and evaporated, leaving the soaps behind.

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[1] Betts, "Chalicosis Pulmonum," Jour. Amer. Med. Assoc., 1900, No. 2.

[2] In the United States cases of albuminous expectoration following thoracentesis have been reported by Pepper, Allen, Pateck, and Riesman. See especially the paper by Riesman, in which a full account of the literature is given. Amer. Jour. Med. Sci., April, 1902, p. 620.

Glycogen has repeatedly been demonstrated in sputa, and may be detected by Ehrlich's method (see Blood).

The sputa of gangrene of the lung and putrid bronchitis have been shown to contain a ferment resembling trypsin. In order to test for this, the sputa are extracted with glycerin; the examination is then continued as described in the chapter on the Examination of Cystic Contents.

The myelin granules, as I have already indicated, consist largely of protagon, lecithin, and cholesterin.

CHAPTER VII.

THE URINE.

GENERAL CHARACTERISTICS OF THE URINE.

Appearance.—Normal urine, just voided at an ordinary temperature, is either perfectly clear or but faintly cloudy, owing to the fact that the acid and normal salts present are all soluble in water. It may be stated, as a general rule, that whenever a urine *freshly passed* presents a distinct cloudiness, some abnormality exists.

When allowed to stand for a time a light cloud develops, which gradually settles to the bottom, constituting the so-called *nubecula* of the ancients. Examined under the microscope this is found to contain a few round, granular cells, somewhat larger than normal leukocytes, the so-called *mucous corpuscles*, and a few pavement-epithelial cells, derived from the bladder or genital organs. Chemically the nubecula probably consists of traces of mucus.

When kept for twenty-four hours at an ordinary temperature, crystals of uric acid are frequently observed in addition to the above elements, usually presenting the so-called whetstone form. If, however, the temperature at which the urine is kept approaches the freezing point, the entire volume becomes cloudy, owing to precipitation of acid urates, as these are much less soluble in cold than in warm water; on standing they gradually settle to the bottom of the vessel and form what is known as a *sediment*, while the supernatant fluid again becomes clear.

If kept still longer exposed to the air, at the temperature of the room, the entire volume of urine again becomes cloudy, owing to a diminution of its normal acidity, the result being a precipitation of ammonio-magnesium phosphate, calcium phosphate, and still later, when the urine has become alkaline, of ammonium urate.

Gradually a heavy sediment, containing these salts in addition to the constituents of the primitive nubecula, forms at the bottom of the vessel; the supernatant fluid, however, remains cloudy. On microscopic examination it will be seen that this cloudiness is due to the presence of enormous numbers of bacteria.

The changes which take place in a normal urine when allowed to stand at ordinary temperature may be tabulated as follows:

1. Urine clear, no sediment; reaction acid.
2. Urine slightly cloudy, owing to development of the nubecula; reaction acid.

Nubecula
- Mucous corpuscles,
- Pavement-epithelial cells.

3. Urine clear; the nubecula has settled; reaction acid.

Sediment
- Mucous corpuscles,
- Epithelial cells,
- Uric acid crystals,
- A few bacteria.

4. Urine cloudly, owing to the precipitation of phosphates; reaction faintly acid or alkaline.
5. Urine cloudy, owing to the presence of bacteria; reaction alkaline.

Sediment
- Bacteria,
- Mucous corpuscles,
- Epithelial cells,
- Triple phosphates,
- Tricalcium phosphate,
- Ammonium urate.

Color.—The color of normal urine may vary from a very light yellow to a brownish red, the particular shade depending essentially upon the specific gravity, becoming lighter with a diminishing and darker with an increasing density. Pathologically the same rule holds good, except in diabetes, in which a very high specific gravity is generally associated with a very light color. The reaction of the urine also exerts a marked influence upon its color, an acid urine being more highly colored than an alkaline urine, which can be readily demonstrated by allowing a specimen of acid urine to become alkaline, and by treating an alkaline urine with dilute hydrochloric or acetic acid. At the same time it may be said that every urine darkens slightly on standing, the reaction remaining acid.

The various shades observed in normal urines may be grouped under the following headings:

1. Pale urines vary from a faint yellow to a straw color.
2. Normally colored urines are of a golden or an amber yellow.
3. Highly colored urines present a reddish-yellow to a red color.
4. Dark urines vary between brownish red and reddish brown.

As these shades may occur in both normal and pathological urines, definite conclusions cannot, as a rule, be drawn from mere inspection. A very pale urine indicates an excess of water, which may be normal, but may also occur in such diseases as chronic interstitial nephritis, diabetes mellitus, diabetes insipidus, hysteria, and the various anemias; it is further seen during convalescence from acute febrile diseases, while a highly colored urine, though also occurring in health, may indicate the existence of a febrile process.

The normal color of the urine is probably owing to the presence of several pigments, which are most likely closely related to each other and to hematin.

In addition to these colors others may be observed at times which are either pathological or accidental—*i. e.*, due to the presence of certain drugs. The former are, on the whole, of greater importance to the physician than those mentioned above, as more definite conclusions can be drawn from their presence. The most important pathological pigments are:

1. Blood-coloring matter. The color in such cases may vary from a bright carmine to a jet black, the exact shade depending upon the quantity of blood-coloring matter present, upon changes that the blood may have undergone either before or after being passed, and also upon the presence of the pigment in solution or contained in red corpuscles.

2. Biliary coloring matter. The color here varies from a greenish yellow to a greenish brown.

Among the accidental abnormalities in color are those due to the presence of substances like carbolic acid and its congeners, santonin, etc. A milky-colored urine is observed in cases of chyluria.

As the recognition of the causes of such alterations, normal, pathological, and accidental, largely depends upon a more detailed study of the individual pigments, this subject will be dealt with more fully farther on. (See Pigments and Chromogens.)

Odor.—The odor of the urine is usually of little significance. Normally it resembles that of bouillon, and in some cases that of oysters; it is probably due to the presence of several volatile acids. The odor of urines undergoing decomposition is characteristic and has been termed "the urinous odor of urine," an ill-chosen term, as this odor is always indicative of an *abnormal* condition.

The ingestion of asparagus, onions, oil of turpentine, etc., produces characteristic odors.

Consistence.—Urine, while normally fluid and but slightly viscid, may in disease acquire a marked degree of viscidity, which becomes especially apparent upon attempting its filtration; the liquid passes through the paper with more and more difficulty, and finally clogs its pores altogether. In old, neglected cases of cystitis it may be ropy and gelatinous.

Quantity.—The quantity of the urine is normally subject to great variations, the amount eliminated in the twenty-four hours being influenced by that of the fluid ingested, the nature and quantity of the food, the process of digestion, the blood pressure, the surrounding temperature, sleep, exercise, body weight, sex, age, etc.

It is easy to understand, then, why figures given by different observers in different countries should vary considerably. Salkowski, in Germany, thus gives 1500 to 1700 c.c. as the normal amount; v. Jaksch, in Austria, 1500 to 2000 c.c.; Landois and Sterling, in

England, 1000 to 1500 c.c.; Gautier, in France, 1250 to 1300 c.c. In the United States I have found an average secretion of from 1000 to 1200 c.c. in the adult male, and 900 to 1000 c.c. in the adult female. It is thus seen that the secretion of urine is greatest in Germany and Austria, where the body weight and ingestion of liquids are greater than in England, France, and the United States.

Children pass less, but relatively more (considering their body weight) urine than adults.

Women pass somewhat less than men.

During the summer months, when a larger proportion of water is eliminated through the skin and lungs than in cold weather, less urine is voided. The same occurs during repose, more urine being passed during active exercise, and hence less during the night than during the day.

The amount of urine secreted in the different hours of the day varies greatly, reaching its maximum a few hours after meals. It decreases toward night, and reaches its lowest point in the first hours of the night, after which it begins to rise rapidly until 2 or 3 o'clock in the morning.

The ingestion of large amounts of liquid, of course, increases the daily amount considerably, and 3000 c.c. may be passed under such conditions by an individual in good health, while it may decrease to 800 or 900 c.c. when but little liquid is taken.

After the ingestion of much solid food the secretion of urine is temporarily diminished.

Water containing no salts possesses distinct diuretic properties, as do also beer, wine, coffee, tea, etc.

The most important medicinal diuretics are digitalis, squill, broom, spirit of nitrous ether, juniper, urea, etc.

Pathologically the amount of urine varies within wide limits. In a given case, moreover, it may be exceedingly difficult to determine whether or not the secretion is within physiological limits. As a general rule, whenever less than 500 c.c. or more than 3000 c.c. are passed some abnormal condition exists, providing all other causes which might lead to the secretion of such an amount can be eliminated.

Clinically we speak of *polyuria* and *oliguria*.

Polyuria.—Polyuria is observed in many diseases, and is present under such varied conditions that a classification is only warrantable upon a hypothetical basis, especially as the causative factors concerned in its production are mostly unknown.

As polyuria is almost invariably associated with diabetes mellitus, its presence in any case should always excite suspicion and lead to a proper examination. The quantity of fluid eliminated in diabetes is usually dependent upon the amount ingested. The excretion of a proportionately large amount of fluid, however, does not necessarily follow the ingestion directly, and retention of a large amount may occur; it has been shown, as a matter of fact, that the diabetic patient excretes liquids with greater difficulty than the healthy subject. At the same time it should be borne in mind that the polyuria in diabetes is not necessarily continuous, and that periods during which a normal or even a subnormal amount is observed may alternate with true polyuria. From 2 to 26 or even 50 liters may be passed within twenty-four hours. Intercurrent diseases of a febrile character may modify the quantity very materially and cause the elimination of a normal or subnormal amount. The cause of the polyuria in diabetes mellitus is unknown.

The polyuria associated with the resorption of large pericardial, pleural, ascitic, and subcutaneous effusions is more readily understood, although the *primum mobile* may be unknown; it depends in such cases entirely upon the presence of excessive quantities of fluid in the bloodvessels.

A form of polyuria which has been termed "epicritic polyuria" is frequently observed during convalescence from acute febrile diseases, and is of prognostic importance. Its occurrence in a given case is regarded by many as a good omen, especially in typhoid fever; still it must not be forgotten that a polyuria may occur after subsidence of the fever, and be followed by a considerable degree of oliguria, and in some cases may precede death. A polyuria of this kind probably always indicates the elimination of waste products which have accumulated in the blood during the course of the disease, but it may, at the same time, be due to the presence of retained water.

Second in constancy is the polyuria associated with granular atrophy of the kidneys. Cases have been reported in which 10,000 c.c. of urine were secreted in the twenty-four hours; 2000 to 4000 c.c. represent the usual amount.

Polyuria is of frequent occurrence early in the course of renal tuberculosis, the increase amounting to one-half of the normal amount.

Very curiously, polyuria may occur also in association with multiple myelomas of the bones and the presence of Bence Jones' albumin in the urine. In one of the cases reported by Hamburger,[1] which I had occasion to study in greater detail from a chemical point of view, 3500 c.c. were voided in the twenty-four hours. The symptom, however, is not constant.

Polyuria, furthermore, has been observed in the most diverse diseases of the nervous system, both functional and organic. It is frequently observed both as a transitory and a more or less permanent symptom in cases of hysteria. Large quantities of a very pale urine are secreted after the occurrence of severe hysterical seizures, but the same may be observed throughout the course of the disease. A similar condition is frequently seen in neurasthenia, migraine, chorea, and epilepsy.

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[1] "Two Examples of Bence Jones' Albuminuria associated with Multiple Myeloma," Johns Hopkins Hosp. Bull., Feb., 1901. C. E. Simon, Amer. Jour. Med. Sci., 1902, vol. cxxiii, p. 954.

Generally speaking, it may be said that a *paroxysmal* polyuria in nervous diseases is associated with functional derangement, while a *continuous* polyuria appears to be connected rather with true organic changes. It has been observed in certain cases of tabes, cerebrospinal and spinal meningitis; during the first stage of general paresis; in association with tumors involving the medulla, the cerebellum, and the spinal cord; in injuries affecting the central nervous system, in Basedow's disease, etc. Cases of idiopathic diabetes insipidus also should probably be classified under this heading. Enormous quantities of urine may be secreted in this disease, which are equalled only by cases of diabetes mellitus, and may at times reach 43 liters per diem.

Oliguria.—Oliguria is, on the whole, more frequent than polyuria, and is met with in almost all conditions associated with a lowered blood pressure. First in order stand those cases of cardiac disease in which compensation has failed, whether the cardiac weakness is primary or occurs secondarily to other diseases—*i. e.*, pulmonary, hepatic, and renal.

The oliguria observed in the so-called continued fevers, notably typhoid fever, is probably also referable to cardiac weakness. It should be remembered, however, that a larger proportion of water is eliminated through the skin and lungs than normally, and that a retention of fluids also undoubtedly occurs which is not due to cardiac weakness; still other factors may be concerned in its production.

The oliguria occurring in acute nephritis and in chronic parenchymatous nephritis in all probability depends largely upon mechanical causes, the increased intra-canalicular resistance in the form of desquamated epithelium and tube casts, as well as the pressure of the exudate upon the bloodvessels obstructing the passage of urine, while the functional activity of the diseased glandular elements is at the same time lowered. Upon mechanical causes, also, depend all those cases of oliguria which are associated with the presence of a stone or tumor pressing upon a portion of the urinary tract.

Oliguria may occur as a nervous manifestation in connection with puerperal eclampsia, lead colic, hysteria, psychic depression, preceding and during epileptic seizures, etc. Whenever there is a diminution in the amount of bodily fluids oliguria is also observed; this is particularly marked in cholera and following severe hemorrhage.

Obstruction to the flow of blood in the vena cava or liver, leading to an increase of venous pressure and a decrease of arterial pressure in the kidneys, likewise results in oliguria, as is seen in atrophic hepatic cirrhosis, acute yellow atrophy, thrombosis of the vena cava and the renal vein, or in cases in which pressure is exerted upon these by tumors, ascitic fluid, etc.

In any case the oliguria may go on to complete anuria, which condition not infrequently precedes death. Anuria may, however, also occur independently of a preëxisting oliguria, as in hysteria.

Specific Gravity.—The specific gravity of normal urine varies between 1.015 and 1.025, corresponding to 1200 to 1500 c.c., viz., the normal amount of urine voided in twenty-four hours. Pathologically, a specific gravity of 1.002 on the one hand and 1.060 on the other may occur, depending upon the amount of solids and fluids present, increasing as the solids increase, the amount of urine remaining the same, and decreasing as the amount of fluid increases, the solids remaining the same. The specific gravity is thus an index in a general way of the metabolic processes taking place in the body.

The necessity of determining the specific gravity of the total amount of urine voided in a given case, and not that of an individual specimen passed during the twenty-four hours, becomes apparent upon considering the variations which may occur in the quantity of solids and liquids ingested during the day. The ingestion of large amounts of fluid would, of course, result in the passage of a correspondingly large quantity of urine within the next few hours, containing but a small amount of solids, and hence presenting a low specific gravity. From such an observation it would be erroneous to infer a diminished excretion of solids for the day, as succeeding specimens would in all probability be passed presenting a higher specific gravity. An observation made upon a specimen taken from the collected urine of the twenty-four hours moreover, can only then convey a correct idea if the total quantity is known.

From the specific gravity the amount of solids can be calculated with sufficient accuracy for clinical purposes by multiplying the last two decimal points by 2, the number obtained indicating the amount of solids in 1000 c.c. of urine.

From the rule, that the specific gravity of a urine is inversely proportionate to the amount of fluid eliminated, it must follow that whatever causes produce oliguria will also produce a high specific gravity, while all those causes which produce polyuria will similarly produce a low specific gravity, with the following exceptions:

1. A diminished amount of urine with a lowered specific gravity occurs in many chronic diseases and toward the fatal termination of acute diseases, indicating a defective elimination of solids.
2. The same may be observed in certain cases of edema.
3. Following copious diarrhea, vomiting, and sweating.
4. A high specific gravity is associated with polyuria in diabetes mellitus.

Unfortunately the determination of the specific gravity and the solids contained in urine does not furnish as valuable information any cases as would be expected *a priori*. This is largely owing he fact that the organic constituents of the urine have a lower ific gravity than the inorganic salts, and especially the chlorides, ch are usually present in considerable amount. It thus not infre- ntly happens that the nitrogenous constituents are considerably eased, while the specific gravity is relatively low, owing to the ence or a diminution in the amount of chlorides. In other words, while the specific gravity may be regarded as a fair index of the total amount of solids excreted, its increase or decrease furnishes no information as to the nature of the constituents causing such a change.

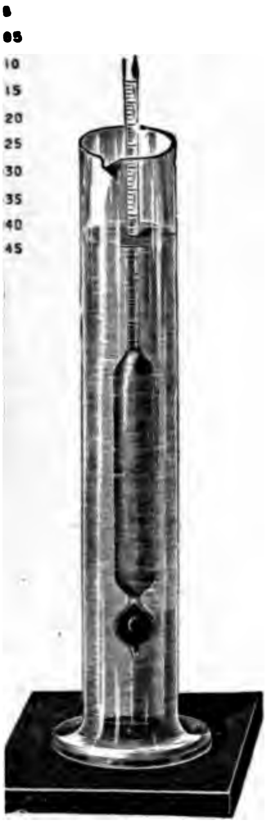

FIG. 131.—Urinometer.

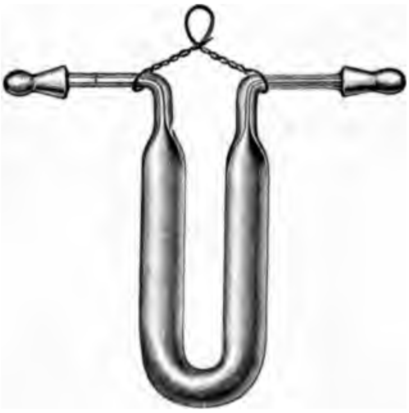

FIG. 132.—The pyknometer.

Determination of Specific Gravity.—The specific gravity of the urine is most conveniently determined by means of a hydrometer indicating degrees varying from 1.002 to 1.040. Such instruments, constructed especially for the examination of urine, are termed *urinometers* (Fig. 131). A good instrument should have a stem upon which the individual isions are at least 1.5 mm. apart, and each division should corre- nd to 0.5 degree.

rinometers may also be purchased which are provided with a rmometer. Every instrument should be carefully tested by com- ison with a *standard* hydrometer.

n order to determine the specific gravity in a given case a cylindrical el is nearly filled with urine and the urinometer *slowly* introduced, the reading being taken at the lower meniscus as soon as the instrument has come to rest.

Precautions: 1. The urinometer must be given ample room, and the reading should never be taken when the instrument touches the sides of the vessel, as owing to capillary attraction it is otherwise raised, causing the reading to be too high.

2. The instrument must be perfectly dry and clean before being used, and should never be allowed to "drop" into the urine, as otherwise the weight of the instrument is increased by adhering drops of fluid, and the reading is too low.

3. Any foam upon the surface of the urine should first be removed by means of a piece of filter paper, as it interferes with the accuracy of the reading; bubbles of air adhering to the instrument, and thereby elevating it, should be removed with a feather.

4. The specific gravity should always be determined in specimens taken from the twenty-four-hour urine.

5. If the quantity of urine is too small to determine its specific gravity with a urinometer, the following method may be employed:

About 50 c.c. of urine are measured into a small bottle provided with a ground-glass stopper, or into a pyknometer like the one pictured in Fig. 132, and accurately weighed. The weight of the urine divided by its volume gives the specific gravity, which must, however, be corrected for the temperature of the urine. If accuracy is required, such corrections should be made in every case, as the specific gravity increases or decreases by 1° for every 3° C. above or below the point for which the instrument is registered, viz., 15° C.

Determination of the Solid Constituents.—As indicated above, the amount of solids can be calculated with a degree of accuracy sufficient for clinical purposes by multiplying the last two figures of the specific gravity by 2; the number obtained indicates the amount of solids in every 1000 c.c. of urine. If greater accuracy is required, the following method may be employed: 5 c.c. of urine, accurately measured, are placed in a watch-crystal containing a little dry sand (sand and crystal having been previously weighed); this is placed over a dish containing concentrated sulphuric acid, and under the receiver of an air pump which has been made perfectly air-tight by thoroughly lubricating the ground-glass edge of the bell with mutton tallow and applying the bell with a slightly grinding movement to the ground-glass plate. The receiver is now exhausted and the urine allowed to remain in the vacuum for twenty-four hours, when the bell is again exhausted and left for twenty-four hours longer; at the end of this time the crystal is weighed, the difference between the two weights obtained indicating the amount of solids in 5 c.c. of urine, from which the percentage and total amount are readily calculated.

The slight loss of ammonia which results when this method is employed scarcely affects the accuracy of the result.

Reaction.—The reaction of the twenty-four-hour urine is, as a rule, acid; individual specimens, passed in the course of the same twenty-our hours, may be either alkaline, acid, or amphoteric.

It has been generally held in the past that the acid reaction of ormal urine is due to the presence of diacid phosphates. But it was assumed also that monosodium phosphate was present at the same ime. Folin[1] has shown that this assumption is not correct, that the phosphates in clear urine are all of the monobasic kind, and that the cidity of such urines is ordinarily greater than the acidity of all the phosphates, the excess being due to free organic acids.

An alkaline urine results when the alkalies exceed the acid equivalents in amount. This may occur under normal conditions (see below), and is then due to a preponderance of monacid over diacid phosphates. An amphoteric urine (red litmus turned blue and blue litmus red) is the outcome, when the acid equivalents of diacid phosphates equal the basic equivalents of the monophosphates; this is essentially an accidental occurrence.

As the alkalinity of the blood increases the acidity of the urine decreases, until an alkaline urine results. The degree of the alkalinity of the blood, however, depends essentially upon the nature of the food and the secretion of the gastric juice, viz., the hydrochloric acid. The ingestion of vegetable food, rich in salts of organic acids, which become oxidized in the body to the carbonates of the alkalies, will result in the passage of an alkaline urine, for the alkalies thus formed when absorbed into the blood are more than sufficient to neutralize completely all the acids present, and the elimination of neutral sodium phosphate alone takes place. In the case of animal food the reverse holds good. The alkaline carbonates here formed are not sufficient to neutralize the excess of acids, and diacid phosphate of sodium is hence eliminated in large quantity.[2]

As the alkalinity of the blood is increased during the secretion of the acid gastric juice, it may frequently happen, especially following the ingestion of a large amount of food, that an alkaline urine is voided. If this does not take place, the acidity of the urine is at east diminished, but increases again during the process of resorption.[3]

If an acid urine is allowed to stand exposed to the air for a certain length of time, its degree of acidity gradually diminishes and the reaction finally becomes alkaline. At the same time the urine becomes cloudy and deposits a sediment, which consists of ammonio-magnesium phosphate, $MgNH_4PO_4+6H_2O$, neutral calcium phosphate, $Ca_3(PO_4)_2$, and still later contains ammonium urate, $C_5H_2(NH_4)_2N_4O_3$, in addition to the constituents of the primitive nubecula

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[1] Amer. Jour. of Physiol., Feb., 1905, vol. xiii.

[2] E. Salkowski u. J. Munk, Virchow's Archiv, 1877, vol. lxxvi, p. 500.

[3] Quincke, Zeit. f. klin. Med., vol. vii.

—*i. e.*, a few mucous corpuscles and pavement epithelial cells. The entire volume of urine, moreover, remains cloudy, owing to the presence of innumerable bacteria. The odor becomes extremely disagreeable and distinctly "urinous." In short, "ammoniacal decomposition" has occurred. This has been shown to depend upon the action of certain bacteria, notably the Micrococcus ureæ and the Bacterium ureæ, which are present in the air.[1] These organisms cause the decomposition of the urea found in every urine, with the formation of ammonium carbonate, according to the following equations:

$$CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3$$
$$(NH_4)_2CO_3 = 2NH_3 + H_2O + CO_2.$$

An alkaline urine, the alkalinity of which is not due to ammoniacal fermentation, however, but to other causes, as indicated above, may, of course, undergo the same change as an acid urine; but it is necessary to distinguish sharply between these two varieties of alkaline urines, as the recognition of the cause of the alkalinity is very often most important in diagnosis. The distinction is readily made by fastening a piece of sensitive red litmus paper in the cork of the bottle containing the urine. If the alkalinity of the urine is due to the presence of ammonia, the litmus paper will turn blue, but soon changes to red when exposed to the air; while a urine the alkalinity of which is due to the presence of fixed alkalies will turn red litmus paper blue *only when immersed in the urine*, the change in color at the same time persisting.

As ammoniacal decomposition can also occur within the urinary passages, it is important, whenever an alkaline reaction due to the presence of ammonia is observed, to test the urine at once upon being voided, or, still better, to procure a portion with a catheter. Such urines are frequently seen in cases of cystitis the result of paralysis, urethral stricture, gonorrhea, etc. In this connection it is interesting to note that whereas in old, neglected cases of cystitis an alkaline reaction is frequently observed, Brown has shown that in the great majority of cases of cystitis, both acute and chronic, and also in those of pyelitis and pyelonephritis, the urine is acid.[2]

An intensely acid reaction is observed in almost all concentrated urines, especially in fevers, in certain diseases of the stomach associated with a diminished or suspended secretion of hydrochloric acid, in gout, lithiasis, acute articular rheumatism, chronic Bright's disease, diabetes, leukemia, scurvy, etc. Whenever a very acid urine is secreted for a considerable length of time, the possibility of renal irritation and the formation of concretions should be borne in mind.

An alkaline urine the alkalinity of which is not owing to the pres-

[1] W. Leube, "Ueber die ammoniakalische Harngährung," Virchow's Archiv, 1885, vol. c, p. 555.

[2] T. R. Brown, Johns Hopkins Hosp. Rep., 1901, vol. x, p. 11.

ence of ammonia, but to fixed alkali, is observed in certain cases of debility, especially in the various forms of anemia, following the resorption of alkaline transudates, the transfusion of blood, frequent vomiting, a prolonged cold bath, etc. It may also be due to the ingestion of certain drugs, viz., salts of the organc acids and alkaline carbonates, the former being transformed into the latter, as has been mentioned. An increase in the degree of acidity may similarly take place after the ingestion of mineral acids.

Of interest is the observation of Pick[1] that in twenty-four to forty-eight hours after the crisis in pneumonia the urine shows a marked decrease in its acidity, becoming neutral or even alkaline. This phenomenon, which was observed in 31 out of 38 cases, persists for a day or a day and a half, and then the acidity returns. In all likelihood the change is due to absorption of the large amounts of sodium which are present in the exudate.

An increase in the acidity of the urine upon standing has repeatedly been observed, and is probably due to the formation of new acids from preëxisting acid-yielding substances, such as certain carbohydrates, alcohol, etc., which have undergone fermentation. This phenomenon is frequently observed in diabetic patients.

A decrease in the acidity of normal urine upon standing, however, is the rule, owing to a gradual decomposition of sodium urate by the acid sodium phosphate, acid sodium urate, and, later on, uric acid resulting, which are thrown down as a sediment in consequence of the diminished acidity of the urine, and which, hence, no longer influence its reaction. This is shown in the equations:

1. $NaH_2PO_4 + C_5H_2Na_2N_4O_3 = Na_2HPO_4 + C_5H_3NaN_4O_3$.
2. $NaH_2PO_4 + C_5H_3Na\,N_4O_3 = Na_2HPO_4 + C_5H_4N_4O_3$.

Determination of the Acidity of the Urine.—Folin has shown that the methods of Freund, Lieblein and Nägeli, which have heretofore been largely in use, are inapplicable and has suggested the following procedure:

Folin's Method.—The total acidity which indicates the acidity due to diacid phosphates and free organic acids is first determined as follows: 25 c.c. of urine are treated with 1 or at most 2 drops of ½ per cent. alcoholic solution of phenolphthalein and 15 to 20 grams of powdered potassium oxalate. The solution is shaken for about a minute and titrated *at once* with decinormal sodium hydrate solution until a faint, yet distinct pink color is obtained. The flask should be shaken during the titration, so as to keep the solution as strong as possible in oxalate. The acidity is expressed in terms of decinormal sodium hydrate solution for the total amount of urine of twenty-four hours. The total acidity is termed T.

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[1] "The Urine in Pneumonia," Münch. med. Woch., 1898, No. 17.

In a second specimen the total phosphates are then determined, the value being termed P (see Phosphates). The result is expressed in terms of decinormal acid, viz., alkali as above (1 c.c. $\frac{n}{10}$ = 7.1 mgrms. of P_2O_5). T minus P then indicates the acidity due to uncombined organic acids (O. A.), and the difference the mineral acidity (M. A.).

It may happen that the acidity calculated from the total phosphates is greater than the titrated acidity; in that case practically no free organic acids are present and the titrated acidity represents the amount of phosphates present in the diacid form. Urines of this kind are turbid, unless they are also free from calcium (Folin).

As average normal value for the acidities of the total bulk of twenty-four hours' urine Folin obtained 617 (c.c. $\frac{1}{10}$ n. acid, viz., alkali), of which 304 was referable to mineral and 313 to organic acidity. The corresponding minimal and maximal values were T 554, viz., 669; M. A. 204, viz., 417; O. A. 252, viz., 378.

With this method a complete revision of all the work previously done will be necessary. The older results given above have reference only to the old method of titration with a one-tenth normal solution of sodium hydrate.

LITERATURE.—Folin, Amer. Jour. of Physiol., 1903, vol. ix, p. 265; and ibid., 1905, Feb., pp. 53 and 54, and ibid., p. 102.

Determination of the Mineral Acidity or the Excess of Mineral Acids or Bases.—Folin's method may be employed instead of determining all the different metals and acids separately as Bunge, Magnus Levy and others have done.

To 25 c.c. of urine in a platinum dish is added from 0.3 to 0.5 gram of potassium carbonate, weighed within an accuracy of two-tenths of a mgrm. The solution is evaporated to dryness, and the residue ignited, when perfectly dry, over a radial burner, using at first a very low heat, and at no time allowing the dish to become more than faintly red hot. The dish is heated at this temperature for one hour, then cooled, when 10 c.c. of hydrogen peroxide are added and evaporated. The dried residue is ignited as before for one hour. It is dissolved in an excess of tenth normal hydrochloric acid and water (50 to 75 c.c. $\frac{n}{10}$ HCl), transferred to an Erlenmeyer flask, boiled to remove carbonic acid, and cooled. One or two drops of phenolphthalein solution and a few crystals of neutral potassium oxalate (to precipitate the calcium) are added, and the solution titrated as usual. The ammonia, the acidity of the hydrogen peroxide, and the acidity of the organic sulphur (neutral and ethereal, 8 grams of which are taken to represent 1 c.c. tenth normal acid) must be subtracted from the result given by the direct titration. These values, as well as the acidimetric value of the potassium carbonate, must be separately determined.

This procedure gives very reliable results, if proper care is used in the evaporation and the burning of the urine. It is to be used only when the actual excess of mineral acids above that necessary for the neutralization of the mineral bases is to be estimated, or when the total amount of organic acids in urine (whether free or combined with bases) is to be determined (Folin).

CHEMISTRY OF THE URINE.

General Chemical Composition of the Urine.—A general idea of the chemical composition of the urine and the quantitative variations of the individual components may be formed from the following table, which I have constructed from analyses made in my laboratory. The individuals from which the urines were obtained were adults, and their general mode of life, as regards diet, exercise, etc., was that of the average American city dweller. In addition, the following substances may be encountered under pathological conditions: serum albumin, serum globulin, albumoses, mucin (nucleoalbumin), glucose, lactose, inosit, dextrin, biliary constituents, viz., bile acids and bile pigments, blood pigments, melanin, leucin, tyrosin, oxybutyric acid, allantoin, fat, lecithin, cholesterin, acetone, alcohol, Baumstark's substance, urocaninic acid, cystin, hydrogen sulphide, and still others.

Analysis of Urine.

Water	1200–1700	grams.
Solids	60.0	
Inorganic solids	25.0–26.0	“
Sulphuric acid (H_2SO_4)	2.0– 2.5	“
Phosphoric acid (P_2O_5)	2.5– 3.5	“
Chlorine (NaCl)	10.0–15.0	“
Potassium (K_2O)	3.3	“
Calcium (CaO)	0.2– 0.4	“
Magnesium (MgO)	0.5	“
Ammonia (NH_3)	0.7	“
Fluorides, nitrates, etc.	0.2	“
Organic solids	20.0–35.0	“
Urea	20.0–30.0	“
Uric acid	0.2– 1.0	“
Xanthin bases	1.0	“
Kreatinin	0.05–0.08	“
Oxalic acid	0.05	“
Conjugate sulphates	0.12–0.25	“
Hippuric acid	0.65–0.7	“
Volatile fatty acid	0.05	“
Other organic solids	2.5	“

Quantitative Estimation of the Mineral Ash of the Urine.—In order to estimate the amount of mineral ash in the urine the following method may be employed: 50 c.c. of urine are evaporated to dryness in a weighed porcelain dish, at a temperature of 100° C., and then heated, while covered, over the free flame until gases cease to be evolved, care being taken not to heat too strongly in order to avoid sputtering. The residue is taken up with distilled boiling water, and, after standing, filtered through a Schleicher and Schüll filter, the weight of the ash of which is known. The dish and the contents of the filter are well washed with hot water. Filtrate and washings are set aside and the dish and filter dried in the oven at 115° C. The filter is now placed in the dish and slowly incinerated. So soon as the ash has turned white the filtrate and washings are placed in the same dish, evaporated at 100° C., and then carefully heated over the free flame. Upon cooling in the desiccator (Fig. 133) the dish with its contents is weighed, the difference between its present and previous weight indicating the quantity of ash contained in 50 c.c. of urine.

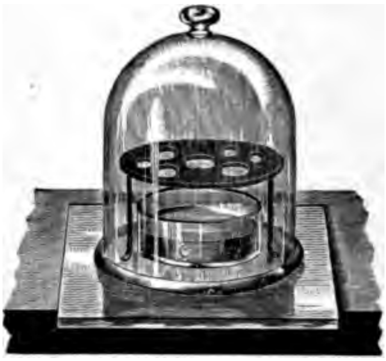

FIG. 133. -Desiccator.

Precautions: 1. Care should be taken to allow the dish to become faintly red only for a moment, as some of the chlorine is otherwise volatilized. Some phosphoric acid may also escape, and too strong a heat, moreover, may cause the transformation of sulphates into sulphides, the organic material present acting as a re ducing agent.

2. If the organic ash is not completely incinerated, it is best to allow the dish to cool and then to moisten the ash with a few drops of dilute sulphuric acid, when the heating is continued.

The Chlorides.

The chlorides which are excreted in the urine are derived from the food. As they are thus present in a much larger amount than all other inorganic salts combined, and in quantity more than sufficient to supply the needs of the body economy, the relatively large amount of chlorides found in the urine under physiological conditions, as compared with the other inorganic constituents, is readily explained.

Of the alkalies in the urine, sodium in combination with chlorine exists in greatest amount, and for clinical purposes it is most convenient to calculate the total quantity of chlorides in terms of sodium chloride; a small proportion also occurs combined with potassium, ammonium, calcium, and magnesium.

From 11 to 15 grams of sodium chloride, representing the total quantity of chlorine, are normally eliminated in the twenty-four hours, the amount depending, of course, directly upon that contained in the food ingested. If the amount of nourishment is diminished, a decrease in the elimination of the chlorides is observed. If this is carried to the point of starvation, the chlorides disappear almost entirely from the urine, the traces remaining being derived from the body fluids. The latter retain tenaciously a certain amount, which differs but slightly from that normally present. If at this stage food containing sodium chloride is again taken, a portion will be retained in the body until the original equilibrium is restored. A similar retention may be observed for a few days following the ingestion of large quantities of water, which causes an increased elimination of chlorides.

This tenacity on the part of the body in retaining sodium chloride is strikingly seen when the potassium salt is substituted for the sodium salt; in this case the amount of the sodium in the serum of the blood will be found to vary very slightly.

It has also been shown that the excretion of sodium chloride can be increased very materially by the ingestion of potassium salts, notably the neutral potassium phosphate (K_2HPO_4). This is supposed to decompose the sodium chloride present in the serum, with the formation of potassium chloride and neutral sodium phosphate, which are both eliminated as foreign material; a point is finally reached, however, when the sodium chloride ceases to be excreted.

This provision of the economy, in virtue of which an increase in the elimination of the salt is followed by its retention, and a previous retention by an increased elimination, is supposed to be intimately associated with the albuminous metabolism of the body. It may be stated, as a general rule, that any increase in the amount of circulating albumin will be followed by an increased elimination of chlorides, these having been previously retained by the albuminous bodies in consequence of the great affinity which exists between them. At the same time the elimination of the chlorides is influenced by the quantity of urine excreted, increasing and decreasing with its volume.

Pathologically the excretion of the chlorides may vary within wide limits, diminishing on the one hand to zero and increasing on the other to 50 grams or more in the twenty-four hours. A marked diminution, which in some cases may go on to a total absence, was formerly thought to be pathognomonic of acute croupous pneumonia.[1] More modern investigations, however, have shown that such a condition occurs to a greater or less degree in most acute febrile diseases, such as scarlatina, roseola, variola, typhus and typhoid fevers, recurrens, and acute yellow atrophy. Intermittent fever appears to be an exception to this rule; usually it is true the chlorides are diminished, but not to the extent seen in the other diseases mentioned. They have, moreover, been found to increase during and sometimes immediately after a paroxysm, this increase being, of course, followed by a corresponding diminution.

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[1] Rettenbacher, Wien. med. Zeit., 1850, p. 373. Heller, Heller's Archiv, 1844, vol. i, p. 23.

The chlorides are diminished in all acute and chronic renal diseases associated with albuminuria.[1] In this connection it is interesting to note that in cases of nephritis associated with edema and other transudates the withdrawal of the chlorides from the food results in marked improvement and in some cases in the complete disappearance of the effusion.

In all cases of carcinoma of the stomach, and in chronic hypersecretion associated with dilatation, a decrease is observed, which in certain cases of hypersecretion and hyperacidity, the result of gastric ulcer, may go on to a total absence.[2]

In anemic conditions the chlorides are likewise diminished, as also in rickets. In melancholia and idiocy a striking decrease is observed; in dementia, chorea, and pseudohypertrophic paralysis this is less marked.

A total absence has been noted in pemphigus foliaceus, and a considerable diminution in the beginning of impetigo, as also in chronic lead poisoning.

The chlorides are found in *increased* amount in all conditions in which retention has previously occurred, chief among these being the acute febrile diseases and cases in which a resorption of exudates and transudates, associated with an increased diuresis, is taking place. A marked increase has been noted in some cases of diabetes insipidus, in which 29 grams have been eliminated in the twenty-four hours.[3] A similar increase may occur in prurigo, in which, in one instance, 29.6 grams were passed in twenty-four hours.[4] In cases of general paresis, during the first stage, an increased elimination goes hand in hand with an increased ingestion of food. In epilepsy the polyuria following the attacks is associated with an increase in the chlorides.

Of drugs, certain diuretics, and some of the potassium salts, as has been mentioned, produce an increase: the chlorine contained in chloroform, whether administered internally or as an anesthetic, is in part excreted in the form of a chloride. Salicylic acid, on the other hand, is said to cause a temporary diminution.

It is of practical importance to note that in acute febrile diseases the diminution in the chlorides appears to vary with the intensity of the disease, a decrease to 0.05 gram pro die justifying the conclusion that the case under oservation is of extreme gravity. It may at times also indicate a preceding attack of severe diarrhea or the formation of exudates of considerable extent. A continued increase, on the other hand, should lead to the conclusion that the patient's condition is improving.

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[1] Röhmann, Zeit. f. klin. Med., 1886, vol. i, p. 513.
[2] Gluzinski, Berlin. med. Woch., 1887, vol. xxiv, 983.
[3] Oppenheim, Zeit. f. klin. Med., vol. vi.
[4] v. Brueff, Wien. med. Woch., 1871, p. 552.

The elimination of the chlorides also furnishes a fair index to the digestive powers of the patient. All other causes which might lead to an increase or decrease being eliminated, an excretion of from 10 to 15 grams indicates a fair condition of the appetite and a normal digestive power, a decrease being associated with the reverse.

An increased elimination of chlorides occurring in cases of edema, and associated with the existence of serous exudates, is always of good prognostic omen, pointing to a resorption of the fluid.

A continued elimination of more than 15 to 20 grams, all other causes being excluded, may be considered as pathognomonic of diabetes insipidus.

Of late attention has been directed to the ratio between the elimination of the chlorides and the total nitrogen. With an ordinary diet this is as 1 to 1 (Salkowski), even though the total amount of chlorides may not amount to 10 to 15 grams, but may be as low as 7 to 10 grams. In disease this ratio may be much disturbed owing to chloride retention (1 Cl to 15 N); a change toward the normal is *cæteris paribus* a favorable sign.

Test for Chlorides in the Urine.—The recognition of the chlorides in the urine is based upon the fact that silver nitrate causes their precipitation. The sliver chloride thus formed is insoluble in nitric acid.

The test is made in the following manner: A few cubic centimeters of urine are acidified in a test-tube with about 10 drops of pure nitric acid, and treated with a few cubic centimeters of silver nitrate solution (1 to 20). The occurrence of a white precipitate indicates the presence of chlorides. An idea may be formed at the same time of the quantity present; the occurrence of a heavy, caseous precipitate points to a large amount. Albumin, if present, must first be removed by boiling, after acidifying the urine with a few drops of dilute acetic acid.

Quantitative Estimation of the Chlorides by the Method of Salkowski-Volhard.[1]—When a solution of silver nitrate acidified with nitric acid is treated with a solution of potassium sulphocyanide or ammonium sulphocyanide, in the presence of a ferric salt, the potassium sulphocyanide first causes the precipitation of white silver sulphocyanide, which, like silver chloride, is insoluble in nitric acid. As soon as every trace of silver is precipitated, it combines with the ferric salt to form ferric sulphocyanide, which is of a blood-red color. If the potassium sulphocyanide solution is of known strength, it is possible to estimate accurately the amount of silver present in the solution, the ferric salt serving as an indicator of the end of the reaction between the silver and the potassium sulphocyanide.

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[1] E. Salkowski, Zeit. f. physiol. Chem., vol. i, p. 16, and vol. ii, p. 379.

Application to the urine: to urine which has been acidified with nitric acid an excess of a silver solution of known strength is added, and the silver not used in the precipitation of the chlorides then estimated as indicated above. The difference between the quantity thus found and the total amount used will be that consumed in the precipitation of the chlorides, from which, knowing the strength of the silver soluion, its equivalent in terms of sodium chloride is readily determined.

Reagents required:

1. A solution of silver nitrate of such strength that each cubic centimeter shall correspond to 0.01 gram of sodium chloride.
2. A solution of potassium sulphocyanide of such strength that 25 c.c. shall correspond to 10 c.c. of the silver nitrate solution.
3. A solution of a ferric salt, such as ammonioferric alum, saturated at ordinary temperature.
4. Nitric acid (specific gravity 1.2).

Preparation of these solutions:

1. As pointed out, the silver nitrate solution is made of such strength that each cubic centimeter shall correspond to 0.01 gram of sodium chloride.

The silver nitrate must be pure, and it is best to use the crystallized salt, and not the sticks wrapped in paper, which always contain reduced silver. In order to test the purity of the salt, about 1 gram is dissolved in distilled water, heated to the boiling point, the silver precipitated by dilute hydrochloric acid and filtered off. When evaporated in a platinum crucible the filtrate should leave either no residue at all or only a very faint one; otherwise it is necessary to recrystallize the salt until the desired degree of purity is reached.

The determination of the quantity to be dissolved in 1000 c.c. of water is based upon the fact that 1 molecule of silver nitrate (molecular weight 170) combines with 1 molecule of sodium chloride (molecular weight 58.5) to form silver chloride and sodium nitrate. As the solution of silver nitrate shall be of such strength that 1 c.c. corresponds to 0.01 gram of sodium chloride, or 1000 c.c. to 10 grams, the quantity to be dissolved in 1000 c.c. is found according to the following equation:

$$58.5 : 170 : : 10 : x, \quad 58.5\,x = 1700, \quad x = 29.059.$$

Theoretically, then, this quantity should be dissolved in 1000 c.c. of water. It is better, however, to dissolve it in a quantity somewhat less than 1000 c.c., such as 900 or 950 c.c., as the silver salt contains water of crystallization and the weighed-off quantity would not represent the exact amount required, but less, the correcting of a solution which is too strong being a much simpler matter than that of a solution which is too weak.

To make this correction, or, in other words, to bring the solution to its proper strength, 0.15 gram of sodium chloride, which has previously been dried carefully by heating in a platinum crucible, is accurately weighed off, dissolved in a little distilled water, and further diluted to about 100 c.c. To this solution a few drops of a solution of potassium chromate are added, when the mixture is titrated with the silver solution. The silver nitrate will first precipitate the sodium chloride, and then combine with the potassium chromate, forming red silver chromate. The slightest orange tint remaining after stirring indicates the end of the reaction. Were the solution of the silver nitrate of the proper strength, exactly 15 c.c. should have been used, as each cubic centimeter shall represent 0.01 gram of sodium chloride. As a matter of fact, less will in all probability be needed, the solution having been purposely made too strong. Its correction then becomes a simple matter, as it is merely necessary to determine the degree of dilution required.

Supposing that 29.059 grams of silver nitrate were dissolved in 900 c.c. of water, and that 14.5 c.c. instead of 15 c.c. had been required to precipitate the 0.15 gram of sodium chloride, it is evident that each 14.5 c.c. of the remaining solution must be diluted with 0.5 c.c. of water. It is, hence, only necessary to divide the number of cubic centimeters of the silver nitrate solution remaining by 14.5; the result multiplied by 0.5 represents the amount of water which must be added in order to bring the solution to the required strength. Hence the rule for the correction of a solution which has been found too strong:

$$C=\frac{N\,.\,d}{n},$$

in which C represents the number of cubic centimeters of water which must be added to the solution remaining; N the total number of cubic centimeters remaining after titration; n the number of cubic centimeters consumed in one titration; and d the difference between the number of cubic centimeters theoretically required and that actually used in one titration.

In the example given the equation would then read:

$$C=\frac{936.5\times 0.5}{14.5}=32.29.$$

32.29 c.c. of distilled water are added to the remaining 936.5 c.c., when the strength of the solution is tested by a second titration. If the solution is found too weak, it is best to make it too strong, and then to correct as described.

2. Preparation of the potassium sulphocyanide solution: as 1 molecule of silver nitrate (molecular weight 170) combines with 1 molecule of potassium sulphocyanide (molecular weight 97), the quantity of the latter to be dissolved in 1000 c.c. of water is found from the following equation:

$$170:97::11.6236:x;\ 170\,x=11.6236\times 97;\ x=6.6.$$

As potassium sulphocyanide is extremely hygroscopic, a solution is made which is too strong, by dissolving about 10 grams of the salt in 900 c.c. of distilled water. In order to bring this solution to its proper strength, 10 c.c. of the silver solution are diluted to 100 c.c.; 4 c.c. of nitric acid (specific gravity 1.2) and 5 c.c. of the ammonioferric alum solution are added, when the mixture is titrated with the potassium sulphocyanide solution; the end reaction is recognized by the production of a slightly reddish color, which persists on stirring. The sulphocyanide solution having been purposely made too strong, it will be found that less than 25 c.c. are needed to precipitate all the silver present. The quantity of water necessary for dilution is ascertained, as above, according to the formula

$$C=\frac{N.d}{n}.$$

3. The solution of ammonioferric alum is a solution saturated at ordinary temperatures, care being taken to ensure the absence of chlorides in the salt, which may be effected, if necessary, by recrystallization.

Method as Applied to the Urine.—10 c.c. of urine are placed in a small stoppered flask bearing a 100 c.c. mark, diluted with 50 c.c. of distilled water, and acidified with 4 c.c. of nitric acid. From a burette 15 c.c. of the standard solution of silver nitrate are added. The mixture is thoroughly agitated and diluted with distilled water to the 100 c.c. mark. The silver chloride formed is filtered off through a *dry*, folded filter into a *dry* graduate; 80 c.c. of the filtrate are placed in a beaker, and, after the addition of 5 c.c. of the ammonioferric alum solution, titrated with the sulphocyanide solution until the end reaction—*i. e.*, a slightly reddish tinge—is seen. If necessary, two such titrations should be made, the sulphocyanide solution being added 1 c.c. at a time in the first, while in the second the total number of cubic centimeters needed to bring about the end reaction, less 1 c.c., are added at once, and then 0.1 c.c. at a time.

The amount of chlorides present in the urine is calculated as follows:

Example.—Total quantity of urine 600 c.c.; 6.5 c.c. of the sulphocyanide solution were required to bring about the end reaction in 80 c.c. of the filtrate; this would correspond to 8.125 c.c. for the total 100 c.c. of filtrate, representing 10 c.c. of urine, as is seen from the equation

$$n : 80 :: x : 100;\ 80\,x = 100\,n;\ x = \frac{100\,n}{80} = \frac{5\,n}{4},$$

in which x represents the number of cubic centimeters corresponding to 100 c.c. of the filtrate, and n the number of cubic centimeters actually used.

These 8.125 c.c. were used in precipitating the silver nitrate not decomposed by the chlorides. As 25 c.c. of the sulphocyanide solution correspond to 10 c.c. of the silver solution, the excess of silver solution in cubic centimeters is found from the equation

$$25 : 10 :: N : x;\ 25\,x = 10\,N;\ x = \frac{10\,N}{25} = \frac{2\,N}{5},$$

in which x represents the excess of the silver solution in cubic centimeters, and N that of the sulphocyanide solution as found according to the equation above, x in this case being 3.25 c.c.

The difference between the total amount of silver solution employed (*i. e.*, 15 c.c.) and the excess (*i. e.*, 3.25 c.c.) indicates the number of cubic centimeters necessary for the precipitation of the chlorides in 10 c.c. of urine. In the case under consideration 11.75 c.c. were employed. As 1 c.c. of the silver solution represents 0.01 gram of sodium chloride, there must have been present in the 10 c.c. of urine 0.1175 gram; in 100 c.c., hence, 1.175 grams, and in the total amount—*i. e.*, 600 c.c. of urine—7.05 grams.

The method described may be employed in the presence of albumins, albumoses, and sugar; the urine, however, must be fresh, so as to ensure the absence of nitrous acid.

Direct Method.[1]—If accuracy is not required, the following method may be employed: 10 c.c. of urine are diluted with distilled water to 100 c.c. and treated with a few drops of a solution of potassium chromate. This mixture is titrated with a one-tenth normal solution of silver nitrate until the end reaction is reached—*i. e.*, a faint orange tinge—which no longer disappears on stirring. The number of cubic centimeters used multiplied by 0.01 will indicate the amount of chlorides present in 10 c.c. of urine.

As uric acid, the xanthin bases, hyposulphites, sulphocyanides, and pigments are also precipitated by the silver nitrate, the end reaction is delayed; moreover, unless the urine is very pale, its recognition may be difficult, and the error thus caused considerable. This is especially true of febrile urines which contain only a small amount of chlorides.

Should iodides or bromides have been taken, these must first be removed, as silver iodide and bromide, which are insoluble in nitric acid, would give too high a value.

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[1] F. Mohr, Lehrbuch d. Titrirmethode, 1856, ii, p. 13.

The Phosphates.

The phosphates occurring in the urine are sodium, potassium, calcium, and magnesium salts of the tribasic acid H_3PO_4. The most important of these, as was pointed out in the chapter on Reaction, is the diacid sodium phosphate NaH_2PO_4, to which the acidity of the urine is in part due. It is owing to the presence of this salt in the urine that the calcium phosphate is held in solution; the fact, at least, that calcium and magnesium phosphate are thrown down when the urine is neutralized would point to this conclusion.

The composition of the phosphates is liable to considerable variation, depending upon the degree of acidity of the urine. As would be expected, diacid sodium phosphate and diacid calcium phosphate are present in an acid urine; in an amphoteric urine, in addition to these there are found disodium phosphate, monocalcium phosphate, and monomagnesium phosphate, while in an alkaline urine trisodic phosphate, neutral calcium phosphate, and neutral magnesium phosphate may be present.

The alkaline phosphates normally exceed the earthy phosphates by one-third, and sodium is combined with by far the greater amount of phosphoric acid, the potassium salt normally occurring in only very small amounts.

In addition to the mineral phosphates, phosphoric acid is excreted also in combination with glycerin as glycerin-phosphoric acid, which need not, however, be considered in a quantitative estimation, as it is present only in traces.[1]

As in the case of the chlorides, the greater portion of the phosphates is derived from the food, while only a small portion is referable to the tissue proteids. But just as the percentage of sulphur varies in the different tissues, so also does that of phosphorus vary; nerve tissue, for example, which is very rich in lecithins and nucleins, yields relatively more phosphorus than muscle tissue.

Not all the phosphoric acid ingested, however, is excreted in the urine, as one-third to one-fourth of the total quantity is eliminated in the feces.

The quantity of phosphoric acid excreted, which normally varies between 2.5 and 3 grams, is thus largely dependent upon the amount ingested, increasing with an animal and decreasing with a vegetable diet.[2] During starvation a considerable increase is likewise observed, referable, no doubt, to an increased destruction of bony tissue, which is very rich in the phosphates of the alkaline earths. In accordance with this view, increased amounts of calcium and agnesium are also seen during starvation. The relation between e excretion of phosphoric acid and nitrogen, normally 1 to 7, changes, oreover, in such a manner that both the absolute and the relative mount of phosphoric acid, as compared with the nitrogen, increases; his leads to the conclusion that in addition to the muscles some other tissue rich in phosphorus and relatively poor in N must suffer during the process, and the only one which could enter into consideration is bone.[1] If at this time food containing phosphorus is again given, a retention will take place, so that the general rule stated in the chapter on Chlorides, that increased elimination is followed by a certain degree of retention, and that a previous retention is followed by an increased elimination, seems to hold good for all the mineral acids found in the urine (see also the chapter on Sulphates).

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[1] Lépine et Eymonnet, Comp.-rend. de la Soc. de biol., 1882.

[2] Zülzer, Virchow's Archiv, vol. lxvi, p. 223.

An increased elimination is caused also by the ingestion of large quantities of water, which is followed by a certain degree of retention.

Observations on the phosphatic excretion during muscular exercise have not given uniform results.[2] Mental exercise appears to cause a diminished excretion of the alkaline phosphates and an increased elimination of the earthy phosphates.[3] The latter also takes place during sleep.

In disease the total amount of phosphates may either be increased or diminished.

A *diminished* elimination is observed in most cases of acute febrile disease, such as pneumonia, typhoid fever, typhus fever, recurrens, during a paroxysm of intermittent fever, etc. The degree of diminution is usually proportionate to the severity of the disease, reaching its lowest figure as death approaches. Such a state of affairs may, at first sight, appear paradoxical in view of what has been said above of the effects of tissue destruction upon the elimination of phosphates. It is necessary, however, to distinguish sharply between an increased production and an increased elimination; in all probability a retention occurs analogous to that of the chlorides, which may be observed under the same conditions. It has been supposed that the phosphates set free during the process of tissue destruction are utilized in the building up of new leukocytes, and an increase in these is actually noted in some of the diseases mentioned. A diminished excretion of phosphates is, however, not always observed, and an increased elimination may occur in certain cases. In fatal cases this condition may persist even until the time of death. It is very difficult to give a satisfactory explanation of this fact at the present time. The phenomenon, in typhoid fever at least, appears to be connected with the intensity of the nervous manifestations, and Robin concludes that here an increased elimination during the fastigium is an unfavorable omen, while an increase during defervescence warrants a favorable prognosis. A similar decrease in the phosphates has also been observed in pulmonary phthisis associated with high fever.[1]

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[1] **Zülzer**, loc. cit.

[2] **Fleischer** u. Penzoldt, Virchow's Archiv, vol. lxxxvii, p. 210.

[3] **Mariet**, Compt.-rend. de la Soc. de biol., 1884.

Very interesting and important is the diminshed excretion of phosphates associated with acute and, to some extent also, with chronic nephritis, amyloid degeneration of the kidneys, and the anemias, in which an actual insufficiency on the part of the kidneys in the elimination of these salts appears to exist.[2]

A diminished or, at least, no increased excretion is usually seen in certain diseases of the bones, such as osteomalacia. This may depend either upon a retention or an elimination through other channels. The *earthy* phosphates especially are found in greatly diminished amount, or may even be absent altogether in certain cases of nephritis. A similar condition is observed in acute and chronic rheumatism.

The data regarding the phosphatic elimination in nervous and mental diseases are, on the whole, scanty and by no means uniform.

During attacks of hysteria major, in contradistinction to epilepsy, in which an increased elimination takes place, the phosphates are diminished, the degree of diminution being generally proportionate to the intensity of the attack, increasing again together with the other urinary constituents with the subsequent increase in the diuresis.[3]

In chronic lead poisoning a diminution to one-third of the normal quantity may occur. Very low figures have been noted in Addison's disease, in acute yellow atrophy (in which even a total absence may occur), and in certain cases of hepatic cirrhosis. In gout the phosphoric acid curve follows that of the uric acid quite closely, decreasing before the onset of the acute symptoms and then rising and reaching its maximum about the third day (see Uric Acid).[4]

An *increased* elimination of phosphates, on the other hand, amounting in some cases to 7 or even to 9 grams in the twenty-four hours, has been described by Teissier, of Lyon, under the name of *phosphatic diabetes*, the patient presenting various symptoms commonly seen in diabetes mellitus; sugar, however, is usually absent. Whether or not phosphatic diabetes is a disease *sui generis* is not certain.[5]

In true diabetes mellitus a curious relation has been found to exist between the elimination of sugar and of phosphates, the quantity of the latter rising and falling in an inverse ratio to the amount of sugar. In diabetes insipidus a slight increase is at times found.

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[1] Edlefsen, Schmidt's Jahresber., vol. cxcvi, p. 59.

[2] Fleischer, Deutsch. Arch. f. klin. Med., vol. xxix, p. 129.

[3] De la Tourette and Cathelineau, Centralbl. f. d. med. Wiss., 1889, vol. xlviii, p. 872

[4] T. B. Futcher, Jour. Amer. Med. Assoc., 1902, vol. xxxix, p. 1046.

[5] G. Rankin, "Phosphatic Diabetes," Lancet, March 24, 1900. Teissier, Thèse, Paris, 1876.

Corresponding to the phosphatic retention observed in acute febrile diseases an increased elimination is noted during convalescence.

An increase occurs in the course of cerebrospinal meningitis.

In a case of pseudoleukemia an increase of 7 grams has been noted, while the number of red corpuscles fell from 2,200,000 to 800,000 in four days. To judge from the very careful observations made, there could be no doubt that the high degree of phosphaturia, which was limited to the alkaline phosphates, was referable to this latter source. In leukemia also very high figures are at times observed. Magnus-Levy reports a case in which the patient eliminated about 15 grams of P_2O_5 in fifteen hours. This is exceptional, but other observers have noted 5 to 7 grams on repeated occasions. Considering the extensive destruction of leukocytes and hence of nucleins in leukemia an increased phosphatic excretion appears natural.

In hemorrhagic purpura Edsall[1] noted a large excretion of P_2O_5: 6.192 grams. The same observer states that he has seen this also in chronic leukemia, as soon as *x*-ray treatment is begun; at least in those cases in which there was the characteristic general response, while it did not occur in the negative cases.

While it is apparent that important conclusions cannot be drawn, on the whole, from a knowledge of the absolute phosphatic elimination, unless it be from a study of the relation existing between the excretion of the alkaline and earthy phosphates, a study of the *relative phosphatic excretion* seems to promise more valuable results. According to Zülzer,[2] a definite amount of the phosphates and of the urinary nitrogen is referable to the destruction of albuminous material, so that the relation between the phosphoric acid and the nitrogen must be constant. Another portion, however, is derived from lecithin, one of the most important constituents of nerve tissue, which contains more phosphorus than the albuminous molecule. Whenever, then, the lecithin-containing tissues are more involved in the general metabolism than under normal conditions the relation will no longer be a stable one. This relation which exists between the elimination of nitrogen and phosphoric acid has been termed the *relative value* of phosphoric acid.

The relative value of phosphoric acid in the urine has been calculated as varying from 17 to 20, that of the blood being 3, of muscle tissue 12.1, of brain 44, of bone 426 to 430. This value supposes the absolute value to vary between 2 and 3 grams pro die. It is found according to the following equation:

$$N : P_2O_5 :: 100 : x; \text{ and } x = \frac{100 \cdot P_2O_5}{N},$$

in which N indicates the amount of nitrogen actually observed, P_2O_5 the amount of phosphoric acid in the same specimen of urine, and x the amount of P_2O_5 corresponding to 100 grams of N. By observing this relative value a much better idea may be formed of the metabolic processes taking place in the body in disease than from a mere expression of the absolute phosphatic value.

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[1] Amer. Jour. Med. Sci., October, 1905.

[2] Loc. cit.

In acute febrile diseases the relative as well as the absolute diminution of the phosphates has been ascribed to a retention, they being possibly utilized in the building up of white blood corpuscles. In the course of these diseases oscillations in the relative value are frequently observed; during convalescence the relative as well as the absolute value again rises.

In accordance with these considerations a diminished relative excretion of phosphoric acid should be expected in all cases associated with a notable elimination of leukocytes through other channels, as in pneumonia, for example, or a storing away of the same, as in cases of empyema. The facts observed are in accord with this view.

A relative decrease has further been noted in the various forms of anemia, conditions of cerebral excitation, and especially preceding an attack of epilepsy. In progressive paralysis following syphilis the relative value, at first low, rises greatly after the administration of potassium iodide, while the excretion of the earthy phosphates is lessened. In chronic cerebral affections, delirium tremens, and acute hydrocephalus a relative decrease has been noted. In mania, during the period of excitement, both the alkaline and the earthy phosphates are found increased, while during the stage of depression, as also in melancholia, the alkaline phosphates are diminished and the earthy phosphates increased. On the other hand, an increase in the relative value has been noted in apoplexy (amounting to 34.3 in one case, two days after an attack), brain tumors, tabes, arthritis deformans (30), pernicious anemia (23.8 to 58), etc.[1]

Of drugs, bromides appear to diminish the absolute amount of phosphoric acid. Cocaine and quinine cause a decrease, and salicylic acid an increase. A relative decrease is produced by the cerebral excitants, such as strychnine, small doses of alcohol, phosphorus, valerian, cold baths, salt-water baths, etc. An opposite effect is produced by the cerebral depressants, such as chloroform, morphine, chloral, large doses of alcohol, potassium bromide, mineral and vegetable acids, prolonged cold baths, Turkish baths, low temperature, etc.

Tests for the Phosphates in the Urine.—The test for the detection of the phosphates occurring in the urine depends upon the precipitation of phosphoric acid by means of ferric chloride as ferric phosphate, which is insoluble in cold acetic acid. The same result may be accomplished by the addition of a solution of uranyl nitrate; this ives rise to the formation of uranyl phosphate, which is also inoluble in acetic acid.

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[1] Zülzer u. Edlefsen, loc. cit.

TEST.—A few cubic centimeters of urine are acidified with a few lrops of acetic acid, and treated with a few drops of a solution of erric chloride (1 part of the officinal solution to 10 parts of water), vhen the occurrence of a yellowish-white precipitate will indicate he presence of phosphates.

If a solution containing an acid phosphate of the alkalies is treated vith an alkaline hydrate, the diacid alkaline phosphate is transformed nto the monacid salt. This is further changed into the normal salt. s the monacid and neutral salts are both readily soluble, the olution remains clear. If at the same time, as in the urine, a soluble iacid phosphate of the alkaline earths is present, this is likewise ansformed into the monacid and finally into the neutral salt; the tter, however, being insoluble, is thrown down.

TEST FOR THE EARTHY PHOSPHATES.—10 c.c. of urine are ndered alkaline with ammonia, when the occurrence of a flocculent recipitate will indicate their presence.

TEST FOR THE ALKALINE PHOSPHATES.—After having removed e earthy phosphates from 10 c.c. of urine, as just described, the ear filtrate is acidified with acetic acid and tested with ferric chlode or uranyl nitrate, as shown above.

The alkaline phosphates may also be detected by treating the mmoniacal filtrate with a few drops of *magnesia mixture* (1 part of rystallized magnesium sulphate, 2 parts of ammonium chloride, 4 arts of ammonium hydrate, and 8 parts of distilled water), when mmoniomagnesium phosphate, which is almost insoluble in ammoium hydrate, will be thrown down.

Quantitative Estimation of the Total Amount of Phosphates. *Principle.* -When a solution of disodium phosphate acidified with acetic acid treated with a solution of uranyl nitrate or uranyl acetate, a dirtyoking precipitate of uranyl phosphate is thrown down. It is pparent that the quantity of phosphoric acid can be estimated ccurately, if the solution of uranyl nitrate or acetate is of known rength.

Solutions required:

1. A solution of uranium nitrate of such strength that 20 c.c. shall orrespond to 0.1 gram of P_2O_5.
2. A solution containing sodium acetate and acetic acid.
3. Tincture of cochineal.

Preparation of these solutions:

1. From the equation

$$2UO.NO_3 + Na_2HPO_4 = (UO)_2HPO_4 + 2NaNO_3$$

is apparent that 2 molecules of uranium nitrate combine with 1 olecule of disodium phosphate to form uranium phosphate and sodium nitrate. The molecular weight of uranium nitrate being 318 and that of disodium phosphate 142, it is seen that 636 parts by weight of the former combine with 142 parts by weight of the latter.

As 20 c.c. of the solution of uranium nitrate shall correspond to 0.1 gram of P_2O_5, 1000 c.c. must be equivalent to 5 grams of P_2O_5. In 142 parts by weight of disodium phosphate there would be present 71 grams of P_2O_5, equivalent to 636 parts by weight of uranium nitrate. The quantity of the latter, then, to be dissolved in 1000 c.c. of water will be found from the equation: 636 : 71 : : x : 5; ad x = 44.78.

44.78 grams of uranium nitrate are weighed off and dissolved in about 900 c.c. of water, the solution being purposely made too strong for reasons pointed out in the chapter on Chlorides. In order to bring this solution to its proper strength it is necessary to titrate with the uranium solution a solution of disodium phosphate[1] of such strength that each 50 c.c. shall contain 0.1 gram of P_2O_5, or 1000 c.c. 2 grams. The molecular weight of $Na_2HPO_4+12H_2O$ being 358, this amount of disodium phosphate in grams is equivalent to 142 grams of P_2O_5; the quantity of P_2O_5 corresponding to 2 grams, in terms of $Na_2HPO_4+12H_2O$, is found from the equation: 358 : 142 : : x : 2; and x = 5.042. This amount of pure, dry, and non-deliquescent Na_2HPO_4 is dissolved in 1000 c.c. of distilled water. If non-deliquescent disodium phosphate is not at hand, about 6 or 7 grams of the salt are dissolved in 1000 c.c. of distilled water; of this solution 50 c.c. are evaporated in a weighed platinum dish, and the residue gently heated, the disodium phosphate being thereby transformed into sodium pyrophosphate, $Na_4P_2O_7$. The molecular weight of $Na_4P_2O_7$ being 266, this corresponds to 142 grams of P_2O_5. If the solution is of the correct strength—*i. e.*, containing 0.1 gram of P_2O_5 in 50 c.c. of water—the residue should weigh 0.1873 gram, as is seen from the equation : 142 : 266 : : 0.1 : x; and x=0.1873. Supposing, however, that the residue weighs 0.1921 gram, it is manifest that the solution is too strong, and must be diluted, the degree of dilution being ascertained according to the equation: 0.1873 : 1000 : : 0.1921 : x; and x = 1025; *i. e.*, 1000 c.c. of the solution must be diluted to 1025 c.c. to make it of the proper strength.

In the case given, 50 c.c. were used; the 950 c.c. are then diluted with the amount of water found from the equation: 1000 : 1025 : : 950 : x; and x=973.75. Having thus obtained a solution of disodium phosphate of such strength that each 50 c.c. shall contain 0.1 gram of P_2O_5, this is titrated with the uranium solution, which has been made too strong, in order to determine the amount of water that must be added to the latter. To this end, a burette is filled with the uranium solution; 50 c.c. of the disodium phosphate solution are treated with a few drops of the tincture of cochineal and 5 c.c. of the acetic acid mixture (see below). This mixture is heated in a beaker, and as soon as the boiling point has been reached titrated with the uranium solution until a trace of a greenish color is noticed in the precipitate which does not disappear on stirring. This point having been accurately determined by means of a second titration, the number of cubic centimeters of distilled water with which the remaining solution must be diluted is determined according to the formula: $C=\frac{N.d}{n}$, in which C represents the number of cubic centimeters which must be added, N the number of cubic centimeters remaining after the test titration, n the number of cubic centimeters consumed in one titration to bring about the end reaction, and d the difference between the number of cubic centimeters used in one titration and that theoretically required.

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[1] A solution of chemically pure crystallized monopotassium phosphate can also be used for standardization (Sutton's Volumetric Analysis, 8th ed., p. 316).

The amount of distilled water necessary for dilution is now added and the solution again tested, when 20 c.c. will correspond to 0.1 gram of P_2O_5.

2. The acetic acid mixture is prepared by dissolving 100 grams of sodium acetate in a little water, adding 30 grams of glacial acetic acid and diluting the whole to 1000 c.c.

3. Tincture of cochineal. This may be prepared as follows: A few grams of cochineal granules are digested at ordinary temperatures with 250 c.c. of a mixture of 3 volumes of water and 1 volume of 94 per cent. alcohol. The solution is then decanted and ready for use. The residue may be utilized in the preparation of a fresh supply of the tincture.

Application to the Urine.—50 c.c. of clear filtered urine are treated with 5 c.c. of the acetic acid mixture, the object being to transform any monacid sodium phosphate present into diacid sodium phosphate, and to neutralize any nitric acid that may be formed during the titration, as otherwise the nitric acid would cause a partial solution of the precipitated uranyl phosphate. A few drops of the tincture of cochineal are added, when the mixture is heated to the boiling point and titrated as described above. Two titrations are usually required.

Should it be desired to use potassium ferrocyanide as an indicator, the uranium solution must have been standardized with the same indicator, as errors will otherwise arise. The technique is simple. A number of droplets of the potassium ferrocyanide solution (about 5 per cent.) are placed on a piece of white filter paper. After every addition of the uranium solution to the boiling urine a droplet of the mixture is placed upon the ferrocyanide stain. The end reaction is indicated by the occurrence of a brown color.

The results are calculated as follows: Supposing 15 c.c. of the uranium solution to have been used, the corresponding amount of P_2O_5 in 50 c.c. of urine is found from the equation: $20:0.1::15:x$; and $x = 0.075$. The percentage amount would, hence, be $0.075 \times 2 = 0.15$. Supposing the total amount of urine to have been 2000 c.c., the elimination of P_2O_5 would correspond to 3 grams.

The presence of sugar and albumin does not interfere with the method.

Separate Estimation of the Earthy and Alkaline Phosphates.—If the alkaline and earthy phosphates are to be determined separately, the total amount of P_2O_5 is estimated in one portion of the urine, while the P_2O_5 in combination with the alkaline earths is determined in another, as follows: 200 c.c. of filtered urine are made strongly alkaline with ammonium hydrate and set aside, covered, for several hours, when the earthy phosphates thus preciptated are collected on a filter, washed with dilute ammonia (1 to 3), and then transferred to a beaker, with the aid of a little water containing a few drops of acetic acid, by perforating the filter. They are then dissolved with as little acetic acid as possible, diluted to 50 c.c. with distilled water, and titrated with the uranium solution as described. The difference between the total amount of P_2O_5 and the amount thus obtained indicates the quantity of alkaline phosphates present.

Removal of the Phosphates from the Urine.—Whenever it is necessary to remove the phosphates from the urine in the course of an analysis, as is frequently the case, the urine is rendered alkaline by the addition of the hydrate of an alkaline earth and precipitated with a soluble calcium or barium salt. They may also be precipitated by means of neutral or basic lead acetate, in which case the excess of lead is removed by means of hydrogen sulphide or dilute sulphuric acid.

The Sulphates.

The sulphuric acid found in the urine is derived essentially from the albuminous material which is constantly broken down in the body, a very small portion only of the inorganic sulphates being referable to the mineral constituents of the food. As was pointed out in the chapter on Reaction, sulphuric acid is constantly produced in the body, and, coming into contact with the so-called neutral phosphates present in almost all the tissues, transforms these into acid phosphates, both appearing in the urine. The alkaline carbonates, which are derived from the organic salts ingested by a process of oxidation, are also attacked by the sulphuric acid.

As the amount of food ingested is gradually diminished a point is reached when the body most tenaciously holds any alkaline salts that may still be present. A new source for the neutralization of the acid is then found in the ammonia, which would otherwise have been eliminated as urea.

While the greater portion of the sulphuric acid excreted in the urine is found in the form of mineral sulphates, about one-tenth of the total amount may be shown to be in combination with aromatic substances belonging to the oxy-group; most important among these are the salts of phenol, indoxyl, and skatoxyl.

Indoxyl and skatoxyl, as will be shown later, are derived from indol and skatol, which, together with phenol, are formed during the process of intestinal putrefaction. Their amount increases and decreases with the degree of putrefaction, and hence serves as an index of its intensity.

The mineral sulphates have been termed preformed sulphates in contradistinction to the others, which are known as conjugate or ethereal sulphates. In the following pages the former will be designated by the letter *A*, the conjugate sulphates by the letter *B*, and the total sulphates as $A+B$.

The amount of $A+B$ excreted in the twenty-four hours by a normal individual varies between 2 and 3 grams, the ratio of *A* to *B* being as 10 to 1.[1]

From what has been said, it is apparent that the elimination of sulphates is largely dependent upon the degree of albuminous destruction taking place in the tissues and fluids of the body, and hence to a certain extent upon the quantity of proteid material ingested, the mineral sulphates occurring in such small amount in the food as scarcely to affect the quantity excreted. Secondarily, the degree of intestinal putrefaction plays a *role.* During starvation $A+B$ is diminished, this diminution affecting *A* especially; in some cases *B* may be considerably increased.[2]

An increase in the elimination of the total sulphates is observed, as would be anticipated, in all cases in which an increased tissue destruction is taking place, as in acute febrile diseases. It must be remembered, however, that the quantity excreted is then not always greater than during convalescence, the diet remaining the same. Here, as elsewhere, in urinary studies, it is necessary to distinguish between a relative increase and an absolute decrease. In pneumonia and acute myelitis the highest figures have been observed, the increased elimination during the febrile period being especially marked:[3]

	Fever diet.		Full diet.
	Fever.	No fever.	No fever.
Pneumonia	3.51 gm.	1.47 gm.	2.25 gm.
Acute myelitis	2.62 gm.	1.52 gm.	2.33 gm.

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[1] v. d. Velden, Virchow's Archiv, vol. vii, p. 343.

[2] Clare, Inaug. Diss., Dorpat, 1854.

[3] P. Fürbringer, Virchow's Archiv, vol. lxxiii, p. 39.

During convalescence the excretion of the sulphates is diminished, a retention analogous to that of the chlorides and phosphates taking place. In contradistinction to the latter salts, it is in all probability not the mineral matter proper that is demanded by the body, but the sulphur-containing albuminous material.

A considerable elimination of $A+B$ has also been observed in leukemia, in which an average of 2.46 grams is excreted, as compared with 1.51 grams by a healthy individual receiving the same amount and kind of food. In one case of acute leukemia 5.8 grams were eliminated on the day preceding death.[1]

In diabetes mellitus, diabetes insipidus, esophageal carcinoma, progressive muscular atrophy, pseudohypertrophic paralysis, and eczema an increased elimination has likewise been observed, while in chronic renal diseases a diminished excretion is the rule.

A study of the elimination of the *conjugate sulphates* and of the relation existing between A and B in disease is still more important than that of the total sulphates; but in both cases the data available are scanty, and further observations are urgently needed. v. Noorden regards the elimination of more than 0.3 gram of conjugate sulphates in the twenty-four hours as excessive, the patient being on an ordinary mixed diet.

The conjugate sulphates, as would be expected, are increased in all cases of increased intestinal putrefaction.[2] In coprostasis the result of carcinoma the ratio of the preformed to the conjugate sulphates, normally 10, may diminish enormously. In one case, reported by Kast and Baas,[3] it fell to 2, but rose to 7 and 8, and finally to 9.5 and 15 after an artificial anus had been established. I have observed a drop to 1.5 in a case of volvulus of ten days' standing. H. Baldwin notes a case of pernicious vomiting of pregnancy in which the factor $A : B$ was 1.9; following abortion it fell to 4 and a little later to 5.4. Biernacki[4] found an increase in the elimination of conjugate sulphates amounting to from 0.15 to 0.5 gram pro die in cases of chronic parenchymatous nephritis, going hand in hand apparently with a decrease in the secretion of hydrochloric acid by the stomach; the normal amount, according to his observations, varies from 0.1973 to 0.2227 gram. In one case B fell from 0.4382 to 0.1505 during the administration of hydrochloric acid, to increase again to 0.4127 upon its discontinuance.

In accord with these observations are those of Wasbutzki and

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[1] Ebstein, Deutsch. Arch. f. klin. Med., vol. xliv, p. 346.

[2] Blumenthal has called attention to the fact that this is not necessarily the case, and that an acid fermentation may occur in lieu of the formation of aromatic products. He hence suggests that at times it may be necessary to estimate the volatile fatty acids also.

[3] Münch. med. Woch., 1888.

[4] Deutsch. Arch. f. klin. Med., vol. lxix.

Kast.[1] The former found an increased elimination of *B* in cases of intense bacterial fermentation taking place in the stomach, while hydrochloric acid was either totally absent or present in greatly diminished amount. A diminished elimination was observed in cases of intense torular fermentation, hyperchlorhydria existing at the same time. In the absence of hydrochloric acid a normal or even a slightly diminished amount was observed in cases of intense acid fermentation, lactic acid and butyric acid being present in large quantities.

By neutralizing the gastric juice with large doses of sodium bicarbonate Kast was able to bring about a marked increase in the elimination of *B*, the ratio $A : B$ having fallen from 10.3 to 16.1 to 2.9 to 6.1. Personal observations have led me to the same conclusion.[2] (See also chapter on the Aromatic Bodies.)

In obstructive jaundice the excretion of *B* is likewise increased; it returns to the normal as soon as the permeability of the biliary passages has again become established. The total sulphates were found diminished in cases of non-obstructive jaundice.[3] In Böhm's[4] cases of catarrhal jaundice the excretion of conjugate sulphates varied between 0.4 and 0.7 gram. Of interest in this connection are the observations of Müller,[5] who notes the elimination of 0.29, 0.24, and 0.28 gram of conjugate sulphates on three consecutive days in a case of total obstruction of the biliary duct in consequence of a stone. The patient during this period was on a milk diet, and there can be little doubt that the low values are here referable to the pure lactic acid producing organisms crowding out the colon bacilli. On a meat diet the same patient passed 0.48 and 0.51 gram. Other observers have obtained less constant results in their cases of catarrhal jaundice. In cases of hepatic cirrhosis and malignant disease of the liver Eiger[6] and Hopadze[7] found increased amounts of conjugate sulphates.

In cases of diarrhea $A+B$, as well as *B*, is diminished, while $A:B$ is increased.

Of drugs, large doses of morphine, potassium bromide, sodium salicylate, and antifebrin appear to cause an increased elimination of the total sulphates, while alcohol slightly diminishes the excretion.

Most important are the observations which have established a diminished excretion of the conjugate sulphates following ingestion of the terpenes and camphor, Karlsbad and Marienbad water, which latter two, however, at first cause an increase. Kefir, in doses of from 1 to 1.5 liters pro die, has proved a most excellent remedy with which to combat this type of intestinal putrefaction. Injections of tannic acid and of a saturated solution of boric acid apparently produce little effect unless the dose is so large as to cause symptoms of poisoning.

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[1] Kast. Festsch. z. Eröffnung d. neuen allgem. Krankenhauses, Hamburg, 1889. Wasbutzki, Arch. f. exper. Path. u. Pharmakol., vol. xxvi.

[2] C. E. Simon, Amer. Jour. Med. Sci., 1895, vol. cx.

[3] Zülzer, Unters. über d. Semiol. d. Harns, Berlin, 1884.

[4] Deutsch. Arch. f. klin. Med., 1901, vol. lxxi, p. 73.

[5] Zeit. f. klin. Med., 1887, vol. xii.

[6] Inaug. Diss., St. Petersburg, 1893.

[7] Wratsch, 1893, Nos. 48 to 50.

Tests for the Sulphates in the Urine.—The detection of the mineral and the conjugate sulphates in the urine depends upon the fact that the sulphates of the alkalies are precipitated by barium chloride as insoluble barium sulphate. In the urine the addition of barium chloride at the same time causes a precipitation of the phosphates. These must be kept in solution by the addition of an acid, acetic acid being employed for this purpose whenever the presence of the mineral sulphates is to be demonstrated; hydrochloric acid is inadmissible, as it would cause the decomposition of the conjugate sulphates and set free the sulphuric acid thus held.

To test for the mineral sulphates, a few cubic centimeters of urine strongly acidified with acetic acid are treated with a few drops of a solution of barium chloride, when in their presence a cloud or a white precipitate of barium sulphate will occur.

To test for the conjugate sulphates, 25 c.c. of urine are treated with about the same volume of an alkaline barium chloride mixture (2 volumes of a solution of barium hydrate and 1 volume of a solution of barium chloride, both saturated at ordinary temperatures) and filtered for a few minutes, the preformed sulphates as well as the phosphates being thus removed. The filtrate is then strongly acidified with hydrochloric acid and boiled; the occurrence of a precipitate is referable to conjugate sulphates.

Quantitative Estimation of the Sulphates.—The principle of the method is the same as that just described, the mineral sulphates forming an insoluble precipitate of barium sulphate directly when treated with barium chloride, while the conjugate sulphates do so only upon decomposition with strong hydrochloric acid under the application of heat. In order to estimate the mineral and conjugate sulphates, it is best to determine the total sulphates in one portion and the conjugate sulphates in another, the difference between the two giving the mineral sulphates.

Quantitative Estimation of the Total Sulphates (Folin).—50 c.c. of clear, filtered urine are treated with 5 c.c. of concentrated hydrochloric aid and 5 c.c. of a 4 per cent. solution of potassium chlorate. The mixture is boiled until it is colorless (five to ten minutes) and then treated, while still boiling, with 25 c.c. of a 10 per cent. solution of barium chloride, *drop by drop*. It is kept on a hot-water bath or on an asbestos plate hot (but not boiling) for one-half to one hour. The precipitate is now collected on a Schleicher and Schüll filter, the weight of the ash of which is known (No. 589). Care should be taken never to allow the filter to run dry, and small amounts of hot water must be added to the last cubic centimeters remaining, the final traces being placed upon the filter with the aid of a rubber-tipped glass rod. The precipitate is washed with hot water for a half-hour, and at intervals of a few minutes hot ammonium chloride solution (5 per cent.) is substituted for the water, so that in all five or six additions of ammonium chloride take place in the course of the first twenty minutes' washing. In the end a specimen of the washings must no longer be rendered cloudy, even on standing a few minutes, upon adding a drop of dilute sulphuric acid.

The paper filter is partially dried by folding and pressing gently between filter paper. It is then placed in a weighed crucible, covered with 3 to 4 c.c. of alcohol, and the alcohol ignited. The ash is heated, at first moderately, and almost completely covered with the lid, then only half covered, for five to seven minutes, until the contents of the crucible are white. The crucible, when cooled, is placed in a desiccator and weighed, the difference between the first and the second weighing giving the weight of the barium sulphate obtained from 50 c.c. of urine.

Quantitative Estimation of the Conjugate Sulphates (Folin).—200 c.c. of urine (diluted to a liter if necessary) are treated with 100 c.c. of a 10 per cent. solution of barium chloride, at ordinary temperature. The mixture is set aside for twenty-four hours and the clear supernatant fluid poured into a dry beaker by decanting. This preliminary decantation is necessary, as the barium sulphate precipitate will otherwise go through the paper. The decanted liquid is filtered, 150 c.c. of the clear filtrate, representing 100 c.c. of urine, measured into an Erlenmeyer flask, treated with 10 to 15 c.c. of concentrated hydrochloric acid and 10 to 15 c.c. of a 4 per cent. solution of potassium chlorate. The mixture is then heated to boiling and kept upon a boiling water bath until the barium sulphate has settled and the supernatant fluid is clear. The precipitate is filtered off, washed, dried, and weighed, as described above. The weight thus obtained, deducted from the amount found according to the first method, indicates the amount referable to the mineral sulphates. The molecular weight of $BaSO_4$ being 232.82, that of SO_3 79.86, of H_2SO_4 97.82, and of S 32, the figure expressing the amount of H_2SO_4, SO_3, or S, corresponding to 1 gram of $BaSO_4$, is found according to the following equations:

232.82 : 79.86 : : 1 : x; and $x = 0.34301$. ∴ 1 gram of $BaSO_4$ = 0.34301 gram of SO_3.

232.82 : 97.82 : : 1 : x; and $x = 0.42015$. ∴ 1 gram of $BaSO_4$ = 0.42015 gram of H_2SO_4.

232.82 : 32 : : 1 : x; and $x = 0.13744$. ∴ 1 gram of $BaSO_4$ = 0.13744 gram of S.

To calculate results, it is only necessary to multiply the weight of the $BaSO_4$ by 0.34301, 0.42015, or 0.13744, in order to ascertain the amount of sulphuric acid contained in 50 c.c. of urine, in terms of SO_3, H_2SO_4, or S, respectively.

LITERATURE.—E. Salkowski, Zeit. f. physiol. Chem., 1886, vol. x, p. 346; and Virchow's Arch., 1888, vol. lxxix, p. 551. O. Folin, Amer. Jour. of. Physiology, 1902, vol. vii, p. 152.

Neutral Sulphur.

While the greater portion of the sulphur of the body is eliminated in an oxidized form, small amounts of non-oxidized sulphur bodies are likewise found in every urine. They are collectively spoken of as the neutral sulphur of the urine, and under normal conditions constitute from 12 to 15 per cent. of the total sulphur. The relation existing between the oxidized and the neutral form is, however, inconstant, and varies with the character of the diet, the degree of the proteid metabolism, etc.

Of the nature of the neutral sulphur bodies which occur in *normal* urine, comparatively little is known. At the present time we are acquainted with only two substances belonging to this order, viz., certain sulphocyanides and cysteïn, or a body which is closely related to it. The greater portion of *the sulphocyanides* is undoubtedly derived from the saliva that has been swallowed and absorbed, while a smaller amount may be referable to the trace which is said to be present in normal, uncontaminated gastric juice. The amount of sulphur which is present in this form represents about one-third of the total quantity of the neutral sulphur. *Cysteïn* probably is an intermediary product of the normal metabolism of proteid material. Under normal conditions, however, the greater portion is oxidized to sulphuric acid, and traces only escape to be eliminated as such.

Whether or not *taurocarbaminic acid*, which is a derivative of taurin, is a constant constituent of the urine remains an open question, but is very probable. We know, as a matter of fact, that the amount of neutral sulphur undergoes a distinct diminution in animals when the bile is prevented from entering the intestinal canal by establishing an external fistula. Under pathological conditions a corresponding increase is observed in cases of biliary obstruction, and the amount of neutral sulphur may then reach 40 per cent. of the total sulphur.

Thiosulphates, which are normally present in the urine of dogs and cats, do not occur in human urine under normal conditions. That they may be present in disease has been shown by Strümpell, who found them in a case of typhoid fever. Further observations, however, are wanting.

Another sulphur body belonging to this class, which Abel discovered in the urine of dogs, and which appears to be identical with *ethyl sulphide*, has not been found in the urine of man.

The greatest increase in the amount of the neutral sulphur is observed under certain conditions associated with the appearance of *cystin*. Normally this is not present in the urine, while traces of *cysteïn*, or a closely related substance, as I have already stated, are found. Cystin is of albuminous origin, and as a matter of fact it has been ascertained that all of the loosely combined sulphur and even a portion of the firmly combined form exists in the albumins in the form of the cystin complex. According to Baumann and v. Udranszky, its appearance in the urine is closely connected with the formation of certain diamins, viz., cadaverin, putrescin, and a third diamin which is probably identical with saprin or neuridin. As these diamins were hitherto supposed to result only from the action of certain specific bacteria upon albuminous material, cystinuria was regarded as evidence of a definite infectious process. It is to be noted, however, that cystin itself does not occur in the feces, and that diaminuria does not necessarily accompany the cystinuria. As the result of personal observations I have been led to the conclusion that a causal connection does not exist between the two conditions, and that the diamins in question can be produced in the body tissues directly without the intervention of microorganisms. I regard cystinuria essentially as a metabolic anomaly, the result of a specific insufficiency on the part of certain tissues (liver) of the body. The condition may be temporary, but as a rule it is permanent. It may occur among several members of the same family, but it is noteworthy that no case has been reported in which a parent and child were cystinuric. Consanguinity among parents, which is not infrequently observed in cases of alkaptonuria, is the exception in cystinuria.

In this connection it is interesting to note that according to Löwy and Neuberg[1] the cystinuric is not able to oxidize other mono- and diamino acids when given by the mouth, and that tyrosin and aspartic acid will reappear as such, while lysin and arginin are eliminated as cadaverin and putrescin. Folin and I have not been able to verify this observation so far as tyrosin goes. Abderhalden[2], on the other hand, found tyrosin and leucin in the urine of a cystinuric, who had not been fed any tyrosin as such.

The amount of neutral sulphur which may be met with in cystinuria is subject to wide variation, but not infrequently exceeds 30 per cent. of the total sulphur. As a general rule, the amount of cystin eliminated in the twenty-four hours is less than 0.5 gram. At times, however, larger quantites are found, and on one occasion

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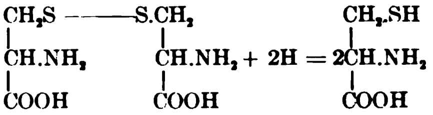
[1] Zeit. f. physiol. Chem., vol. xliii, p. 338.

[2] Abderhalden and Schittenhelm, ibid., vol. xlv, p. 468. C. E. Simon, ibid., vol. xlv, p. 24.

I obtained more than 1 gram. Clinically it is of interst in so far as its continued production may give rise to the formation of calculi.

Unless cystin occurs as a deposit, its presence will scarcely be suspected. The substance, however, may occur also in solution, and it not infrequently happens that attention is first drawn toward its existence in this state owing to the marked odor of hydrogen sulphide which such urines develop on standing (see Hydrothionuria). If acetic acid is then added in excess, the characteristic hexagonal plates may crystallize out. The same result is obtained by allowing the urine to undergo ammoniacal decomposition, as cystin is insoluble in solutions of ammonium carbonate.

Structurally cystin is the disulphide of cysteïn, which latter is α-amino-β-thiolactic acid. On reduction it is transformed into cysteïn according to the equation:



$$\begin{array}{lll} CH_2S\text{———} & S.CH_2 & \quad\; CH_2.SH \\ | & | & \quad\; | \\ CH.NH_2 & CH.NH_2 + 2H = & 2CH.NH_2 \\ | & | & \quad\; | \\ COOH & COOH & \quad\; COOH \end{array}$$

Cystin crystallizes in hexagonal plates which are quite characteristic, and not likely to be confounded with other crystalline elements that may be present in urinary sediments. If doubt should arise, their solubility in ammonia and hydrochloric acid, and their insolubility in acetic acid, water, alcohol, and ether, will lead to their identification.

The quantitative estimation of cystin is rather unsatisfactory, as no method is known which yields reliable results. On the whole, it is perhaps best to determine the neutral sulphur, and to refer the increase beyond its normal value to the presence of cystin.

Quantitative Estimation of the Neutral Sulphur in the Urine.—In one portion of urine the oxidized sulphur, viz., the mineral and the conjugate sulphates, are estimated as described. In a second portion the total sulphur is determined, the difference indicating the amount of the neutral sulphur.

To determine the total amount of sulphur the following method is most conveniently employed:

Method of Höhnel-Glaser (modified by Modrakowsky[1]): 1 or 2 grams of sodium peroxide are placed in a nickel dish, and covered with 50 c.c. of urine, added drop by drop. The fluid is evaporated to a syrup on a water bath, and further treated with 2 to 3 grams of the peroxide, which is added slowly while stirring. As soon as the reaction, which at first is quite vigorous, has subsided somewhat, the dish is removed from the water bath and heated with a small alcohol lamp. If necessary, 1 to 3 grams more of the peroxide are added.

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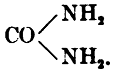
[1] Zeit. f. phys. Chem., 1903, vol. xxxviii, p. 562.

The mass now forms brown drops and finally becomes thick; this ends the reaction. On cooling, the fusion is dissolved in hot water; the solution is filtered and feebly acidified with hydrochloric acid. Barium chloride is then added and the process continued as above described (Estimation of Sulphates).

Literature.—E. Salkowski, Virchow's Archiv, vol. lxvi, p. 313, and vol. cxxxvii, p. 381. Goldmann u. Baumann, "Zur Kenntniss der Schwefelhaltigen Verbindungen des Harns," Zeit. f. physiol. Chem., vol. xii, p. 254. E. Salkowski, Virchow's Archiv, vol. lviii, p. 461. J. Munk, ibid., vol. lxix, p. 354; and Deutsch. med. Woch., 1877, No. 46. O. Schmiedeberg, "Ueber das Vorkommen von Unterschwefliger Säure im Harn," Arch. d. Heilk, vol. viii, p. 425. A. Strümpell, ibid., vol. xvii, p. 390. J. Abel, "Ueber das Vorkommen von Ethylsulfid im Hundeharn," etc., Zeit. f. physiol. Chem., vol. xx, p. 253. (See also Cystinuria and Hydrothionuria.) C. E. Simon, "Cystinuria and its Relation to Diaminuria," Amer. Jour. Med. Sci., 1900, vol. cxix, p. 39. C. E. Simon and M. W. Lewis, "Transitory Cystinuria," ibid., 1902, vol. cxxiii, p. 838. C. E. Simon and D. G. J. Campbell, "A Contribution to the Study of Cystinuria," Johns Hopkins Hospital Bull., 1904, vol. xv, p. 365.

Urea.

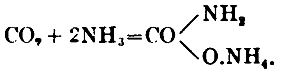
Urea is the most important nitrogenous constituent of the urine, and normally represents from 85 to 86 per cent. of the total amount of nitrogen which is eliminated by the kidneys. Chemically, it may be regarded as carbamide—*i. e.*, as the amide of carbonic acid—and is represented by the formula



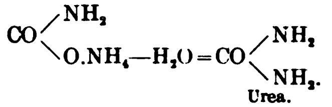
$$CO\begin{cases}NH_2\\NH_2\end{cases}.$$

It is thus a comparatively simple substance, and the question naturally arises: What relation does urea bear to the complex albuminous molecule from which it is derived? According to the older concept of the process of albuminous digestion this leads to the formation of albumoses and peptones, which latter were regarded as a unity and as very complex substances. From these bodies the reconstruction of the albuminous molecule then supposedly took place in the intestinal mucous membrane, whence the resulting albumin found its way into the blood and lymph. Here it existed as so-called circulating albumin, in contradistinction to the organized albumin of the cells. Voit further taught that the circulating albumin is broken down in the tissues at large, through the special activity of the living protoplasm, but without becoming an integral part of the cells before its destruction. Pflüger, on the other hand, took the contrary view according to which the circulating albumin must become part and parcel of the cells before it can undergo katabolic disintegration. In either event it was generally accepted that urea was derived from the tissues of the body at large and to a great extent from the muscles. Regarding the nature of its intermediary antecedents, Drechsel supposed that amino-acids first result by hydrolysis in the tissues, and that ammonia, water, and carbon dioxide are then formed from these by oxidation. Ammonia and carbon dioxide then combine and form ammonium carbamate, which is carried to the liver and is there transformed into urea through loss of water. These stops are represented by the equations:

1. $\underset{\text{Glycocoll.}}{CH_2.NH_2.COOH} + 3O = NH_3 + 2CO_2 + H_2O.$

2. 

$$CO_2 + 2NH_3 = CO\begin{cases} NH_2 \\ O.NH_4. \end{cases}$$

3. 

$$CO\begin{cases} NH_2 \\ O.NH_4 \end{cases} - H_2O = \underset{\text{Urea.}}{CO\begin{cases} NH_2 \\ NH_2. \end{cases}}$$

As a matter of fact it is well known that the ingestion of amino-acids leads to an increased elimination of urea and that the liver plays an important role in its final formation. But there is still much doubt whether amino-acids are formed in the tissues at large to such an extent as the older theories of Voit and Pflüger would demand. It is rather significant that normally they are scarcely ever encountered in the tissues of the mammalian organism, and for some years past there has been a growing tendency to regard ammonium paralactate as the principal form in which the greater portion of the nitrogen leaves the tissues. In the liver this is then supposedly transformed into ammonium carbonate, from which the urea results with the intermediary formation of ammonium carbamate.

This hypothesis has certain facts in its favor. We thus find that after extirpation of the liver in geese the uric acid, which in birds plays the same part as the urea in mammals, disappears and is largely replaced by ammonium lactate. In diseases of the liver, moreover, in which an extensive destruction of the parenchyma is taking place, as in some cases of acute yellow atrophy, in phosphorus poisoning, etc., the elimination of urea is diminished, and in its place a corresponding amount of ammonia in combination with lactic acid is found. In dogs in which the liver has been in part excluded from the general circulation by the establishment of an Eck fistula, and in which the hepatic artery has at the same time been ligated, the elimination of urea is much diminished, while that of ammonia increases rapidly so soon as the first symptoms of illness appear in the animals. From these observations it is apparent also that the synthesis of urea takes place in the liver. This is further proved by the fact that on transfusion of isolated livers of dogs with blood to which ammonium carbonate or ammonium lactate has been added, urea is formed as a result. In other organs of the body this synthesis apparently does not occur, but there is evidence to show that at least a small amount of urea originates elsewhere within the body through processes of hydrolysis. This amount, however, is unquestionably slight. That a fraction, moreover, is formed from uric acid, and in the last instance from the xanthin bases through processes of oxidation, can scarcely be doubted, but this transformation apparently also takes place in the liver.[1]

Of late Folin[2] has formulated a theory of proteid metabolism which in my judgment is more in conformity with our present knowledge of proteid digestion and more satisfactorily explains many questions connected with the subject of nitrogenous metabolism than any other. He distinguishes sharply between tissue metabolism or endogenous metabolism which tends to be constant, and exogenous or intermediate metabolism which is variable. As essential nitrogenous end product in the first instance he regards kreatinin, the elimination of which he finds practically constant for one and the same individual. Urea, according to his conception, is the principal nitrogenous end product in the case of the exogenous metabolism. According to his idea the amino-acids which result on gastro-intestinal digestion, in so far as they are not needed immediately to make up for tissue loss in nitrogen, are at once desamidized in the liver. The non-nitrogenous remainder is then utilized in the formation of fats and carbohydrates, while the amino group gives rise to the formation of urea.

In this manner the presence of the large amounts of ammonium compounds which are found in the portal blood during digestion is well explained. But as Howell remarks, while a portion and perhaps a large portion of the urea arises from this early hydrolysis of the proteids of the food we must admit also that ammonium compounds may be formed in the tissues of the body generally, probably by a similar process of hydrolysis followed by oxidation. This would suggest itself especially under pathological conditions where the amount of urea nitrogen may be in excess of that corresponding to the ingested food.

It has been stated that 84 to 86.6 per cent. of all the nitrogen eliminated in the urine is in the form of urea, the remaining 13.4 per cent. being excreted as uric acid, hippuric acid, kreatinin, xanthin bases, etc. It might hence be supposed that an accurate idea of the degree of tissue destruction could be formed from a quantitative estimation of urea. This, however, is not the case, and especially in pathological conditions, as the quantitative relations existing between the excretion of urea and the remaining nitrogenous constituents are subject to wide variation. In acute yellow atrophy, for example, urea may disappear entirely from the urine, the nitrogen being eliminated in the form of other compounds (leucin, tyrosin, glycocoll, etc.). Whenever it becomes desirable, then, to gain an accurate insight into the degree of proteid destruction or proteid assimilation—in other words, into the nitrogenous metabolism—taking place in the body, it is necessary to resort to a quantitative determination of the total amount of nitrogen excreted by the kidneys; the quantity found is then conveniently expressed in terms of urea. At the same time it is customary to express the amount of proteid tissue which is destroyed, as muscle tissue, as this serves as a fair type of body tissue in general.

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[1] The origin of urea: O. Schultzen u. M. Nencki, Zeit. f. Biol., 1872, vol. viii, p. 124. E. Salkowski, Zeit, f. physiol. Chem., 1879, vol. iv, p. 100. v. Knieriem, Zeit. f. Biol., 1874, vol. x, p. 279. E. Salkowski, Zeit. f. physiol. Chem., 1877, vol i, p. 38. Hoppe-Seyler, Physiol. Chem., 1881, p. 810. Drechsel, Jour. f. prakt. Chem., vol xv, p. 417: vol. xvi, pp. 169 and 180, and vol. xxii, p. 476. M. Hahn, V. Massen, M. Nencki, and J. Pawlow, "La fistula d'Eck," etc., Arch. d. Sci biol. de St. Petersburg, 1892, vol. i.

Seat of formation: W. v. Schröder, Arch. f. exper. Path. u. Pharmakol., 1882, vol. xv, p. 364. W. Salomon, Virchow's Archiv, 1884, vol. xcvii, p. 149. Minkowski, "Ueber d. Einfluss d. Leberextirpation auf d. Stoffwechsel," Arch. f. exper. Path. u. Pharmakol., 1886, vol. xxi, p. 41, and 1893, vol. xxxi, p. 214.

[2] C. Voit, Physiol. d. allg. Stoffwechsels u. d. Ernährung. Hermans' Handbuch d. Physiol., 1881, vol. vi, I. p. 301. O. Folin, Amer. Jour. of Physiol., 1905, vol. xiii.

As 100 grams of lean muscle tissue contain about 3.4 grams of nitrogen, corresponding to 7.286 grams of urea, 1 gram of the latter is equivalent to 13.72 grams of muscle tissue. It is, hence, only necessary to multiply the quantity of urea eliminated in the twenty-four hours, corresponding to the total amount of nitrogen found by 13.72, in order to obtain an idea of the extent of albuminous destruction taking place in the body. If accurate results are desired, it becomes necessary to determine also the amount of nitrogen eliminated in the feces, a knowledge of the quantity in the food ingested being, of course, presupposed.

With all these data given, the nitrogenous metabolism of the body can be accurately controlled.

Example.—A patient eliminates 50 grams of urea in twenty-four hours; these 50 grams correspond to 50 × 13.72—*i. e.*, 686 grams of lean muscle tissue; on the other hand, he ingests an amount of nitrogenous material corresponding to only 10 grams of urea, equivalent to 10 × 13.72—*i. e.*, 137.2 grams of muscle tissue. The difference between the amount ingested and that excreted in this case—*i. e.*, 548.8 grams—must be referable to the destruction of organized albumin.

When the amount of nitrogen eliminated is equivalent to that ingested, *nitrogenous equilibrium* is said to exist. A healthy person is approximately in this condition.

During starvation urea is still eliminated from the body, although in diminshed amount. The question now arises, What happens if at this time an amount of nitrogenous food is given which corresponds exactly in amount to that eliminated? Under such conditions an increased elimination of nitrogen takes place, all of the nitrogen ingested, in addition to that resulting from a breaking down of body tissues, being excreted. The amount of nitrogen referable to the latter source, however, is somewhat less than that eliminated in the total absence of food. Unless starvation has been pushed too far, the body accommodates itself to the amount of food thus given and nitrogenous equilibrium is restored. If more food is allowed an increased elimination results, which again leads to a condition of nitrogenous equilibrium, different levels, so to speak, being possible.

It is apparent, then, that the elimination of urea, and of nitrogen in general, is subject to great variation and depends to a great extent upon the amount ingested. A statement in figures expressing the daily elimination of urea and of nitrogen would, hence, be of very little value, especially in pathological conditions, in which the amount of nitrogen ingested is frequently very small. The elimination of nitrogen should hence always be compared with the amount ingested, for which purpose the tables of König[1] will be found most convenient. At the same time it must be remembered that not all the nitrogen taken into the body as food undergoes resorption, and that a variable amount, which in disease may be considerable, is eliminated with the feces, so that in accurate work this nitrogen also must be taken into account. In order to obviate the tedious estimation of nitrogen in the feces, it has been proposed to determine the standard amount of urea which should appear in the urine of a healthy person under different forms of diet. Such experiments, of course, presuppose the control person to be in a condition of nitrogenous equilibrium, which, from what has been said above, is readily accomplished, as the human body adapts itself with ease to different forms of diet. In general practice, however, such a procedure would be difficult, but here approximate results can be obtained from a parallel estimation of the chlorides. In health the elimination of the chlorides may be placed at about one-half of the urea. Whenever the nitrogen resulting from tissue destruction is in excess of that referable to the proteids ingested, this relation between the excretion of chlorides and urea will be disturbed, as the tissues of the body contain very litte sodium chloride. Whenever the amount of urea is in excess of the normal amount of chlorides, as indicated above, an increased tissue destruction may be inferred, and *vice versa*. If, on the other hand, the chlorides are present in diminished amount, the conclusion may be drawn that a retention of albumins is taking place in the body; this is observed frequently during convalescence from acute febrile diseases.

In most text-books the statement is found that the normal daily elimination of urea varies between 30 and 35 grams. This would imply that a lower amount could be viewed as abnormal. But, as I have pointed out, the urea elimination depends essentially upon the amount of proteid food ingested, and I have long maintained that the consumption of such large amounts of proteids as would lead to the elimination of the quantities stated is totally unnecessary. Every clinician no doubt can recall data which would tend to support this view, and Chittenden and Folin have demonstrated the same fact by numerous observations. As Folin says: the immediate elimination of the greater part of the nitrogen contained in 118 to 130 grams of proteid (Voit's standard) by means of the exogenous katabolism would seem to constitute very strong evidence in favor of the view that the proteid so katabolized can without harm, if not with advantage, be replaced by an equivalent quantity of carbohydrates.

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[1] Chemie d. menschlichen Nahrungs u. Genussmittel, Berlin, 1893.

An *increase in the amount of urea*, and, as a matter of fact, of all the nitrogenous constituents, is observed especially in the acute febrile diseases, notwithstanding the diminished ingestion of nitrogenous material, and is ascribed to the greatly increased tissue destruction.[1] An excretion of 50 grams or more is here frequently observed. Formerly it was thought that the fever itself was responsible for this increased elimination. But this view became untenable when it was shown that the excretion of urea in the beginning of a febrile attack is not proportionate to the height of the temperature, reaching its higest point only when the fever has been continuous for several days. Still larger amounts, moreover, may be eliminated when the fever is abating. Similar observations have since been made. An increased elimination of nitrogen may also be noted in almost every case of ague preceding the onset of the fever. The latter, therefore, cannot be the only factor which causes the increased excretion of urea, and it has been suggested that the cells of the body have lost the power of taking up nitrogen. The question, however, whether this is dependent upon the increase in temperature or the action of certain toxic substances circulating in the blood, or upon both, still remains unanswered.

The large increase in the elimination of nitrogen in febrile diseases is especially striking in those which end by crisis. This is notably the case in pneumonia, in which it may persist for two or three days after the occurrence of the crisis and is then no doubt largely due to the resorption of the exudate.

Apparently, the only exception to the rule that the amount of urea is increased in acute febrile diseases is acute yellow atrophy, in which the excretion of urea is not only greatly diminished, but may cease altogether, its place being taken by other nitrogenous bodies, such as ammonium lactate, leucin, tyrosin, glycocoll, etc.

Among afebrile diseases, in which an increased elimination of urea has been noted, may be mentioned the ordinary forms of diabetes mellitus, in which the highest figures have been obtained, viz., 150 grams or more pro die. This is, in all probability, explained, in part at least, by the ingestion of excessive amounts of proteid food by such patients, but carefully conducted experiments seem to show that a not inconsiderable portion of the urea is directly referable to increased tissue destruction. The cases described by Hirschfeld [1] however, which will be considered later on, form an exception to this rule.

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[1] Vogel, Zeit. f. rationelle Med., N. F., vol. iv, p. 362. Huppert, Arch. d. Heilk., vol. vii, p. 1. Löbisch, Wien. med. Presse, 1889, vol. xxxix, p. 1521. Huppert u. Riesellt, Arch. d. Heilk., vol. x, p. 329. Bauer u. Künstler, Deutsch. Arch. f. klin. Med., vol. xxiv, p. 53.

v. Noorden and Lipman-Wolff have shown that anemia as such is not necessarliy associated with a pathological increase in the albuminous metabolism. But it appears that in pernicious anemia, at least in the bothriocephalus form, there are periods in which an increased albuminous disintegration does occur. According to Rosenqvist,[2] this is far too extensive to be dependent entirely upon the destruction of red corpuscles, but must be associated with changes in other nitrogenous tissues of the body. After the expulsion of the worms a well-marked nitrogenous retention was observed. Similar results were obtained in cases of cryptogenetic pernicious anemia, where periods of markedly increased albuminous disintegration alternated sometimes with such of distinct nitrogenous retention. Rosenqvist concludes that his observations are strongly in support of the theory that cryptogenetic pernicious anemia, like the bothriocephalus form, is also a toxic anemia.

An unusually large output of nitrogen and greatly in excess of the amount ingested is apparently a common feature of acute leukemia. Ebstein records a case in which 62 grams of urea were eliminated in twenty-four hours, and Edsall[3] mentions an instance in which with an intake of only 7.25 grams of nitrogen, 29.534 grams appeared in the urine.

In this connection it is interesting to note that an astonishing increase of the urinary nitrogen occurs on *x*-ray treatment in those cases of chronic leukemia, when the characteristic response so far as the effect upon the spleen and the number of the leukocytes is concerned, takes place, while in the negative cases this is not observed.[4]

In purpura hemorrhagica a notable increase of the urinary nitrogen occurs, apparently without relation to the hemorrhages. Edsall mentions an instance in which the patient, while ingesting not more than 3 to 4 grams, eliminated amounts varying between 14 and 23 grams.

A moderate increase has been found in severe cases of chronic leukemia, scurvy, minor chorea, and paralysis agitans. Observations made in cases of hystero-epilepsy have given rise to conflicting results.

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[1] "Ueber eine neue klin. Form. d. Diabetes," Zeit. f. klin. Med., vol. xix, pp. 294 and 325.

[2] Berlin. klin. Woch., 1901, vol. xxxviii, p. 666.

[3] Amer. Jour., Oct., 1905, p. 589.

[4] Edsall and Musser, Univ. of Penn. Med. Bull., Sept., 1905.

It is claimed, on the one hand, that the excretion of urea is diminished following convulsive seizures of a hystero-epileptic nature, in contradistinction to an increased elimination following true epileptic attacks.

In cases of functional albuminuria associated with an increased elimination of uric acid or oxalic acid, I have observed an increased elimination of urea, and believe that in the treatment of these diseases a systematic study of the excretion of nitrogen is of fundamental importance. The increase is here unquestionably due to the ingestion of excessive amounts of proteids.

Of drugs, an increased elimination is produced by caffeine, morphine, codeine, ammonium chloride, sodium and potassium chlorides, lithium carbonate, following the ingestion of large amounts of water, etc. The data concerning the action of quinine, salicylic acid, cold baths, etc., are conflicting. A large increase has been observed in cases of phosphorus poisoning.

Electricity appears to exert a marked influence upon the excretion of urea, producing an increased elimination.

The *diminished elimination of urea* observed in certain diseases of the liver,[1] notably in acute yellow atrophy, carcinoma, cirrhosis, and even in Weyl's disease, is of special interest, and is in perfect accord with the theory that the liver is the main seat of its production.

As has been stated, urea may disappear altogether from the urine in acute yellow atrophy and also in Weyl's disease, notwithstanding the frequently not inconsiderable degree of fever. In cirrhosis, hyperemia of the portal system has been thought to cause the diminution, which may be increased further by the occurrence of ascites. In short, the factors which may be regarded as causing a diminished elimination of urea in hepatic diseases may be summarized under the following headings:

1. Destruction of hepatic parenchyma.
2. Diminished velocity of the flow of blood through the liver.
3. Insufficient excretion of bile and coincident digestive disturbances.

Whenever there is disease affecting that portion of the renal parenchyma which is concerned especially in the elimination of urea, a diminished amount will be met with, and carefully conducted observations upon the excretion of the various urinary constituents are here of considerable value from a diagnostic as well as a therapeutic standpoint. However, as v. Noorden and others have pointed out, there are periods in the course of a nephritis when the urea output is quite normal.

While, as a rule, the excretion of urea is greatly increased in diabetes mellitus, certain cases, which have been elaborately described by Hirschfeld,[1] must be excepted. His researches have established beyond a doubt that the resorption of nitrogenous material from the intestines may be very much below normal, and with it the elimination of urea. Upon these grounds he has advocated the recognition of a distinct form of diabetes, which is characterized by a comparatively rapid course, the occurrence of colicky abdominal pains before or at the onset of the diabetic symptoms proper, the existence of pancreatic lesions in a certain proportion of the cases, a more moderate degree of polyuria, etc.

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[1] Hallerworden, Arch. f. exper. Path. u. Pharmakol., vol. xii. Weintraud, ibid., vol. xxxi. Stadelmann, Deutsch. Arch. f. klin. Med., vol. xxxiii. Fawitzki, ibid., vol. xlv. Fränkel, Berlin. klin. Woch., 1878 and 1892. v. Noorden, Lehrbuch d. Path. d. Stoffwechsels, p. 287

In mental diseases a diminished excretion of urea has been observed in melancholia and in the more advanced stages of general paresis, while an increase is associated with the increased ingestion of food during the first stage of profound dementia.

Following epileptic, cataleptic, and hysterical seizures, as well as in pseudohypertrophic paralysis, a decrease has been noted by some observers.

In tetanus the elimination of the urea nitrogen is normal or diminished.

In Addison's disease a decrease is commonly noted.

All forms of chronic, non-progressive anemia are associated with a decrease, as are also osteomalacia, impetigo, lepra, chronic rheumatism, etc. In chronic lead poisoning the elimination of urea may be greatly diminished. Little is known of the influence of drugs in bringing about a diminished excretion of urea.

Properties of Urea.—Urea crystallizes in two forms, viz., in long, white needles if rapidly formed, or in long, colorless, quadratic rhombic prisms when allowed to crystallize gradually from its solutions.

At 100° C. it begins to show signs of decomposition; at 130° to 132° C. it melts; and when heated still further it is decomposed into cyanic acid and ammonia, of which the former is immediately transformed into its polymeric compound, cyanuric acid. Biuret is formed as an intermediary product during this decomposition, 2 molecules of urea yielding 1 molecule of ammonia and 1 molecule of biuret. As this substance, obtained on dissolving the residue remaining after all the ammonia has been driven off by careful heating, yields a beautiful reddish-violet color when a drop or two of a very dilute solution of cupric sulphate is added to its solution alkalinized with sodium hydrate, this reaction may be employed as a test in the detection of urea (Biuret test).

Very important is the behavior of urea when treated with a solution of sodium hypochlorite or hypobromite, the most usual method of estimating urea being based upon this reaction, which may be represented by the equation

$$CON_2H_4 + 3NaOBr = 3NaBr + 2N + CO_2 + 2H_2O.$$

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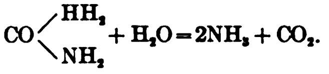
[1] Loc. cit.

In the chapter on Reaction it was pointed out that urine gradually undergoes ammoniacal decomposition when exposed to the air; the ammonia is liberated from the urea according to the equation



$$CO\begin{matrix}\diagup HH_2 \\ \diagdown NH_2\end{matrix} + H_2O = 2NH_3 + CO_2.$$

This decomposition may also be effected by heating a watery solution of urea in a sealed tube to 100° C.

Urea is readily soluble in water, fairly so in alcohol, and insoluble in anhydrous ether and benzol. The aqueous solution of urea is neutral in reaction, but the substance combines with acids, bases, and salts to form molecular compounds.

Urea nitrate, $CON_2H_4.HNO_3$, crystallizes in two different forms: in thin rhombic or six-sided colorless plates, which are frequently observed arranged like shingles one on top of the other when rapidly formed (Fig. 134), while larger and thicker rhombic columns or plates are obtained if the process is allowed to proceed more slowly. Urea nitrate is readily soluble in distilled water, while in alcohol and in water containing nitric acid it dissolves with difficulty. Upon heating, it evaporates without leaving a residue.

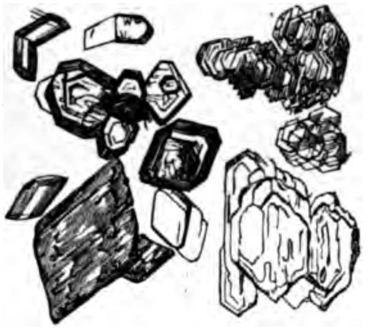


FIG. 134.—Urea nitrate crystals. (Krukenberg, after Kühne.)

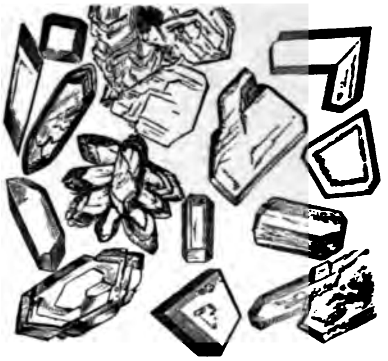


FIG. 135.—Urea oxalate crystals. (Krukenberg, after Kühne.)

Urea oxalate, $CON_2H_4.C_2H_2O_4$, crystallizes in rhombic or six-sided prisms or plates (Fig. 135), which are less soluble in water than the nitrate; in alcohol and in water containing oxalic acid it is only imperfectly soluble.

With mercuric nitrate urea forms three different compounds, according to the concentration of the two solutions, viz., $(CON_2H_4)Hg_2(NO_3)_4$, $(CON_2H_4).Hg_3(NO_3)_6$, and $(CON_2H_4)_2.Hg(NO_3)_2+3HgO$. The latter compound is of special importance, as Liebig's quantitative estimation of urea was based upon its formation.

For the separation of urea from the urine the reader is referred to works on Physiological Chemistry.

Quantitative Estimation of Urea. Hypobromite Method.—The method most commonly used in the clinical laboratory is the one based upon the decomposition of urea into carbon dioxide and nitrogen in the presence of sodium hypobromite. The carbon dioxide thus formed is absorbed by an excess of sodium hydrate in the hypobromite solution, while the nitrogen is set free, and can be collected and measured; the determination of the corresponding amount of urea is then a simple matter.

The hypobromite solution is prepared from two stock solutions. The first of these contain 125 grams of bromine and 125 grams of sodium bromide in 1000 c.c. of water. The second is a 22.5 per cent. solution of sodium hydrate. Immediately before use equal portions of the two solutions are mixed and diluted with one and one-half volumes of water.

The reaction which takes place may be represented by the equation

$$2NaOH + 2Br = NaBr + NaOBr + H_2O$$

Various forms of apparatus, termed *ureometers*, have been suggested for the estimation of urea by this method. One which I have found very satisfactory is represented in Fig. 136. It consists of a burette, *C*, with an ascending rubber tube attached to the reservoir, *B*, which can be raised or lowered as required for the purpose of equalizing the pressure after collection of the gas. A descending tube leads to a wide-mouthed bottle, *A*, which contains the hypobromite solution. This is closed by a tightly fitting rubber stopper, to which a loop of platinum wire is attached carrying a little bucket made of glass or porcelain; this can be swung from its support by inclining the bottle.

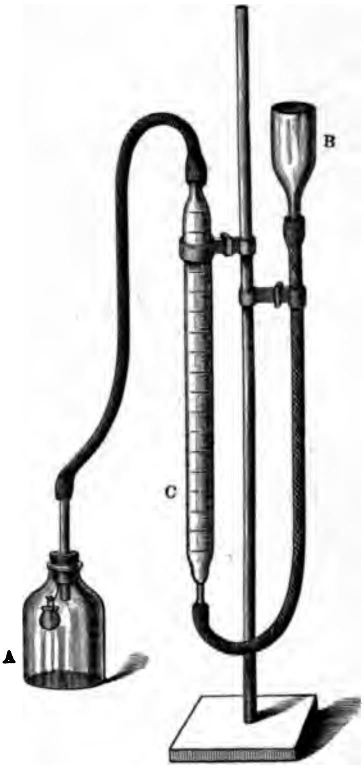


FIG. 136.—The author's ureometer.

METHOD.—The rubber stopper is removed from the bottle *A*, and water poured into *B* until the system *B C* is filled to such an extent that the water level is visible in *B* above the point where the rubber tube is attached. About 25 to 30 c.c. of the hypobromite solution are placed in the bottle *A*, and 2 c.c. of urine in the bucket; this is then attached to the wire loop. The stopper is now adjusted and the water in *B* and *C* brought to the same level, when the first reading is taken. *A* is then inclined until the bucket drops into the liquid below. The nitrogen which is liberated collects in the burette *C*; as a consequence the water falls in *C* and rises in *B*. After twenty to thirty minutes the pressure in *C* is equalized by lowering *B* until the water in both tubes is at the same level. The second reading is then taken, the difference between the two indicating the volume of nitrogen liberated from 2 c.c. of urine at the temperature of the water in *C B*, which, as well as the barometric pressure, should be previously noted.

As the volume of gases is influenced by the temperature, the barometric pressure, and the tension of the aqueous vapor, it becomes necessary, in order that the results reached shall be comparable with those obtained by other observers, to reduce the volume of nitrogen actually noted to a certain standard. This has been placed at 0° C and 760 mercury millimeters pressure, in the absence of moisture. The correction is made according to the following formula:

$V = \frac{v.(B - T)}{760.(1 + 0.00366.t)}$, in which V represents the corrected volume of the gas in terms of c.c., v the volume actually observed, B the barometric pressure in Hgmm., T the tension of the aqueous vapor at the temperature noted, t. The volume of nitrogen observed being thus corrected, the calculation of the corresponding amount of urea is based upon the following considerations: From the formula CON_2H_4 it is apparent that 2 atoms of nitrogen are contained in 1 molecule of urea; in other words, that 28 parts by weight of nitrogen correspond to 60 parts by weight of urea. The equivalent of 1 gram of urea is then found according to the equation: $60 : 28 :: 1 : x$; and $x = 0.46666$. The volume corresponding to 0.4666 gram of dry nitrogen at 0° C. and 760 Hgmm. pressure is 372.7 c.c. It has been found, however, that only 354.3 c.c. of nitrogen are evolved from 1 gram of urea at best when the hypobromite method is employed Knowing that 354.3 c.c. of nitrogen correspond to 1 gram of urea, the amount of urea to which the volume of nitrogen actually observed is referable would then be found according to the equation

$1 : 354.3 :: x : y$; and $x = \frac{y}{354.3}$, in which y denotes the number of cubic centimeters of nitrogen evolved from 2 c.c. of urine, and x the corresponding amount of urea. In order to ascertain the percentage amount of urea it is only necessary to multiply the figure just obtained by 50.

Precautions: (1) The urine must be free from albumin. (2) It should contain only about 1 per cent. of urea—*i. e.*, not more than 0.025

gram in 2 c.c. Whenever a greater amount is noted, therefore, the urine is diluted to the proper degree, due allowance being made in the calculation.

In ordinary clinical work the barometric pressure, as well as the tension of the aqueous vapor, may be ignored.

Of the many other ureometers the one devised by Doremus in the modification of Heinz is most convenient and furnishes very satisfactory results.

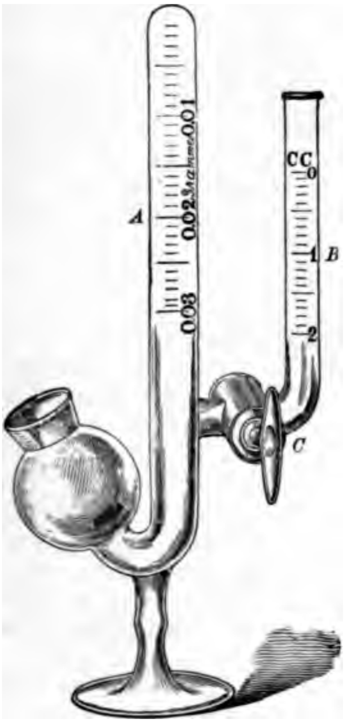


FIG. 137.—Doremus-Heinz ureometer.

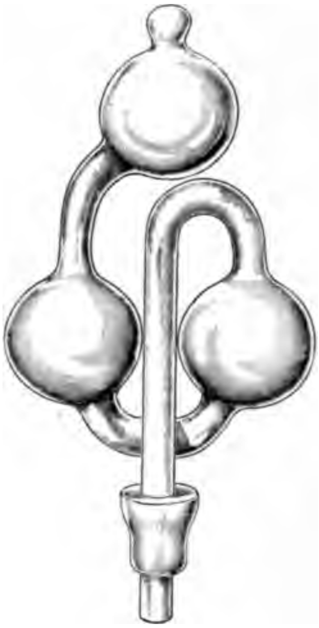


FIG. 138.—Folin's safety-tube.

Its general construction is seen in Fig. 137. A small amount of urine is poured into *B* while the stopcock (*C*) is closed. This is then opened for a moment and again closed, so as to fill its lumen. The tube *A* is washed out with water and filled with the hypobromite solution. The tube *B* is filled with urine to the zero mark, and 1 c.c. (or less, if the urine is concentrated) is allowed to mix with the hypobromite solution[1] in *A*. After all bubbles of gas have disappeared the reading is taken. Each small division corresponds to 0.001 gram of urea and every ten divisions hence to 0.01 gram, for the amount of urine used.

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[1] This is prepared as described on p. 409.

The urine must be free from albumin and should not contain more than 1 per cent. of urea. If necessary it is diluted with water.

In the presence of ammonium compounds the results may be faulty, and in cases where this is suspected it is advisable to resort to more accurate methods, such as that of Folin.

Method of Folin.[1]—This is based upon the following considerations: At a temperature of about 160° C. crystallized magnesium chloride, $MgCl_2.6H_2O$, boils in its water of crystallization. In such a solution urea is quantitatively decomposed into ammonia and carbon dioxide within one-half hour. If the process is carried out in acid solution, the ammonia can subsequently be distilled off after rendering the mixture alkaline, and is then titrated. The corresponding amount of urea is ascertained by calculation. At the same time, however, the preformed ammonia is obtained, and it is hence necessary to eliminate this source of error by a separate estimation of this form. This is conveniently done according to the method which has likewise been suggested by Folin (see below).

METHOD.—3 c.c. of urine when carefully measured with a 5 c.c. pipette graduated in twentieths are placed in an Erlenmeyer flask of 200 c.c. capacity, together with 20 grams of magnesium chloride and 2 c.c. of concentrated hydrochloric acid. (The magnesium chloride usually contains a small amount of ammonia, which must be separately determined.) The flask is closed with a perforated stopper through which a specially constructed safety-tube passes (see Fig. 138).[2] The mixture is now boiled until the drops flowing back through the tube produce a hissing sound on coming in contact with the solution. After this point has been reached the boiling is continued more moderately for about forty-five minutes. Immoderate foaming during this process and the subsequent distillation is guarded against by adding a small piece of paraffin (about the size of two coffee beans).

The solution while still quite hot is carefully diluted to about 500 c.c.—at first by allowing the water to flow drop by drop through the tube; it is then transferred to a 1000 c.c. retort, treated with about 7 or 8 c.c. of a 20 per cent. solution of sodium hydrate, and the ammonia distilled off into a measured amount of a decinormal solution of sulphuric acid. The distillation may be interrupted when about 350 c.c. have passed over (viz., after about sixty minutes). The distillate is boiled for a moment to remove any carbon dioxide which may be present in solution, and on cooling is titrated to determine the excess of acid. Each cubic centimeter of the decinormal ammonia present in the distillate corresponds to 0.003 gram, viz., to 0.1 per cent. of urea.

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[1] Zeit. f. physiol. Chem., vol. xxii, p. 504, and vol. xxxvi, p. 333.

[2] The tube can be obtained from Messrs. Eimer and Amend, of New York.

From this result the amount of preformed ammonia and that present in the 20 grams of magnesium chloride must be deducted.

Estimation of Nitrogen.—For the purpose of estimating the total amount of nitrogen in the urine, the method of Kjeldahl is most conveniently employed.

Kjeldahl's Method.[1] *Principle.*—The organic matter of the urine is decomposed by means of sulphuric acid, when all the nitrogen which is not present in combination with oxygen is transformed into ammonia. After adding sodium hydrate in excess the ammonia is distilled off and received in a known quantity of titrated acid, the excess being retitrated with sodium hydrate. In this manner the amount of ammonia and the corresponding quantity of nitrogen are ascertained, it being remembered that 17 grams of ammonia correspond to 14 grams of nitrogen.

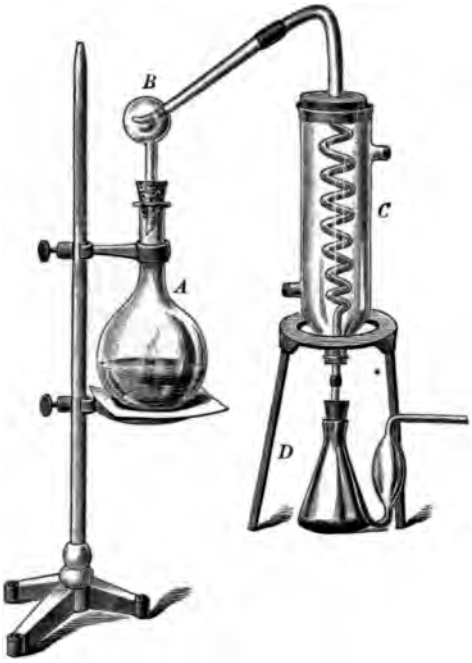

FIG. 139.—Kjeldahl's nitrogen apparatus.

Reagents required:

1. Gunning's mixture. This consists of 15 c.c. of concentrated sulphuric acid, 10 grams of potassium sulphate, and 0.5 gram of

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[1] "Neue Methode zur Bestimmung des Stickstoffes in organischen Körpern," Zeit. f. analyt. Chem., 1883, vol. xxii, p. 366.

cupric sulphate. In the place of Gunning's mixture one of 500 c.c. of concentrated sulphuric acid and 100 grams of phosphoric anhydride may also be employed, and has the advantage that oxidation proceeds more rapidly.

2. A solution of sodium hydrate containing 270 grams in the liter (sp. gr. 1.243).
3. Pulverized talcum or granulated zinc.
4. A one-fourth normal solution of sulphuric acid.
5. A one-fourth normal solution of sodium hydrate.

Apparatus required (see Fig. 139): This consists of a retort of about 750 c.c. capacity (A), which is connected with a Kjeldahl distilling tube (B), and through this with a Städeler condenser (C). The ammonia is received in the nitrogen bulb at D. In addition a Kjeldahl digesting flask of 200 to 300 c.c. capacity is required.

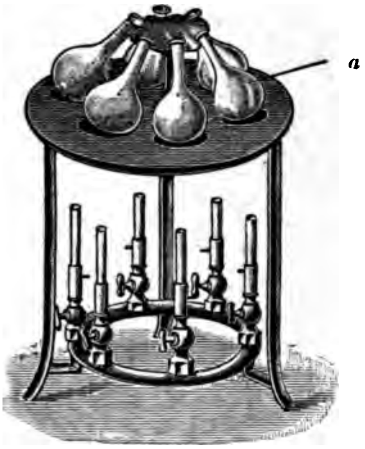

FIG. 140.—Kjeldahl's apparatus for the simultaneous oxidation of six specimens: a, Kjeldahl flasks.

METHOD.—Five or 10 c.c. of urine are placed in the digesting flask and treated with Gunning's mixture. To this end it is best to add the sulphuric acid and cupric sulphate first, to heat until sulphuric acid vapors are given off in abundance, and then to add the potassium sulphate. The heating is continued until the solution becomes entirely clear and almost colorless, the flask being inclined at an angle of about 45 degrees. *Vigorous ebullition should be avoided.* If the sulphuric acid-phosphoric anhydride mixture is to be employed, the urine is first treated with 0.4 gram of mercuric oxide, and 10 c.c. of the acid mixture added. Digestion is then carried on as described. Toward the end of digestion, in either case, it is advantageous to throw a few crystals of potassium permanganate into the fusion, so as to ensure complete oxidation.

Upon cooling, the contents of the flask are transferred to the retort with the aid of a little water, and slowly treated with a moderate excess of the sodium hydrate solution. As a general rule, 40 c.c. for each 5 c.c. of sulphuric acid are sufficient. A little pulverized talcum or a few pieces of granulated zinc are finally added; the retort is connected with the condenser with the interpositon of the distilling tube and the distillation begun. The talcum or zinc serves the purpose of preventing undue frothing and bumping. The distillation is continued until about two-thirds of the solution have passed over. The distillate is received in the nitrogen bulb, which should contain a carefully measured quantity of the one-fourth normal solution of sulphuric acid. As a general rule, 30 c.c. are sufficient. As soon as the distillation is completed the condenser is disconnected, washed out with a small amount of distilled water, and the washings added to the distillate. After the addition of a few drops of tincture of cochineal or dimethyl-amino-azo-benzol the excess of sulphuric acid is retitrated with the one-fourth normal solution of sodium hydrate, and the amount found deducted from the 30 c.c. used. The titration should be continued until every trace of yellow (in the case of the cochineal) has disappeared and a pure rose color is obtained, or, in the case of the dimethyl-amino-azo-benzol, until the last trace of red has disappeared and the solution has turned yellow. The difference multiplied by 0.0035 will indicate the amount of nitrogen present in the 5 or 10 c.c. of urine. The corresponding amount of urea is found by multiplying this figure by 20.

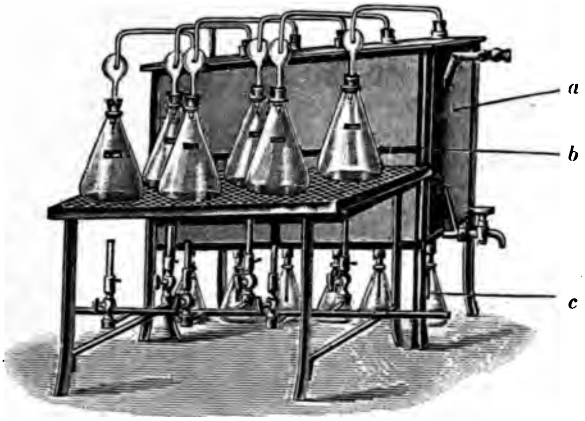

FIG. 141.—Kjeldahl's apparatus for the simultaneous distillation of six specimens: *a*, condenser; *b*, distillation flasks; *c*, receivers.

Whenever several nitrogen determinations are to be carried out daily it is convenient to make use of a special apparatus, which permits of such determinations being conducted at one time. The general plan of the outfit is seen in the accompanying illustrations (Figs. 140 and 141).

Ammonia.

Every urine contains a small amount of ammonia, which normally varies but little, and corresponds to from 4,1 to 4.64 per cent. of the total amount of nitrogen, viz., to about 0.7 gram in the twenty-four hours. It is present in combination with the various acids of the urine, and in all likelihood represents a small amount of the ammonia which has not been transformed into urea, but has been utilized to saturate the affinities of a slight excess of acid, formed during the nitrogenous metabolism of the body, over the available fixed alkalies.

In man an increased elimination of ammonia is observed whenever an increased formation of acids occurs, or whenever a sufficient supply of oxygen is not available. In the latter case, no doubt, the increased elimination is owing to the fact that in consequence of the deficient supply of oxygen the synthetic formation of urea is impeded in the liver. As this organ, moreover, is the principal seat of the synthesis of urea, we can readily understand that extensive parenchymatous degeneration, as in acute yellow atrophy, in phosphorus poisoning, etc., will lead to an increased elimination of ammonia.

In any event, the relative increase of the ammonia is the essential factor, while variations in its absolute quantity are of secondary importance. Some of the results which have been obtained in various diseases are given in the following table:

	Per cent.
Normal values	4.10– 4.64
Febrile diseases	5.72– 6.70
Carcinoma of the liver	6.40–24.50
Liver abscess (actinomycosis)	10.60
Circulatory dyspnea	13.10–32.20
Respiratory dyspnea	6.60–14.30

Abnormally high absolute values are quite constantly observed in diabetes, in which a daily elimination of from 4 to 5 grams may be regarded as common. In a general way the amount of ammonia in cases of diabetes gives an idea of the amount of organic acids; but, as Herter has pointed out, we cannot detect moderate quantities of organic acids in this way. (See Oxybutyric Acid.)

In cases of pernicious vomiting of pregnancy Williams[1] found a large increase of ammonia, up to 20 to 45 per cent., while this does not occur in nervous vomiting and in eclampsia. It is advised that in such cases the uterus be emptied, when the ammonia is said to drop at once.

A slight rise occurs also in normal pregnancy and reaches its maximum during labor.

Very curiously a diminished elimination of ammonia is observed in many cases of nephritis so long as symptoms of venous stasis do not exist.

In a case of pernicious anemia relative amounts, varying between 3.3 and 5.6 per cent., were obtained during the days immediately preceding death.

Quantitative Estimation. Folin's Method.—10 c.c. of urine are diluted to about 45 c.c., treated with a small amount of burnt mag-

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[1] Amer. Jour. of Med. Sci., September, 1906.

(0.5 gram), and boiled for forty-five minutes, the distillate being 'ed in decinormal sulphuric acid through an absorption tube, as the one pictured in Fig. 142. This consists of a glass tube, ɜasuring about 8 mm. in diameter, one extremity of which has blown into the small bulb *b*. By means of a heated platinum five or six holes, each about 1 mm. in diameter, are made in the *c* is a rubber stopper which fits into the second *d*. This is merely a test-tube (2.5 cm. in diam- which has been cut about 7.5 cm. from the r end. About 3 cm. from the upper margin ube is provided with six or seven holes as in *b*. The entire apparatus is directly immersed e decinormal acid and ensures the complete ption of the ammonia in one flask, even if this ins only 5 to 10 c.c. of the acid. The ammonia en determined by titration as above, using in red as indicator; 2 drops of a 1 per cent. on suffice for 200 to 300 c.c. The titration is d to the red point, not to the violet. As a amount of urea, however, is decomposed during rolonged ebullition, it is necessary to ascertain ately the quantity of ammonia which is referable is source. To this end the retort is opened at xpiration of forty-five minutes, and an amount ıter added which is approximately equivalent to of the distillate. The distillation is then con- d for another period of forty-five minutes; the late is received in decinormal sulphuric acid, he ammonia referable to decomposition of the estimated as before. The difference between the results indicates the amount of preformed ammonia that was ıally present; 1 c.c. of the $\frac{n}{10}$ sulphuric acid indicates 0.0017 of ammonia.

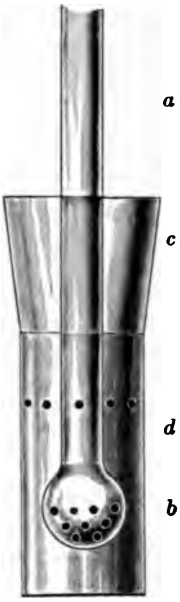


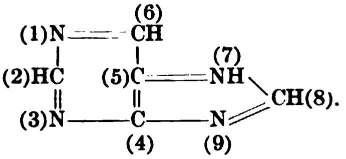
FIG. 142.—Absorption tube.

ıis method is also applicable for the determination of ammonia e blood.

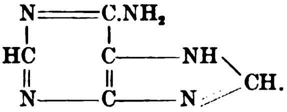
ERATURE.—Hallervorden, Arch. f. exper. Path., vol. xii, p. 237. Stadel- , Deutsch. med. Woch., 1889, p. 942. Michaelis, ibid., 1900, p. 276. O. Zeit. f. physiol. Chem., vol. xxxii, p. 575, and ibid., 1902, vol. xxxvii, .

Uric Acid.

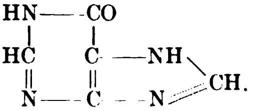
cording to our present views, uric acid, in man, is not formed ıg the decomposition of all albuminous substances, as was for- r supposed, but constitutes a specific product of decomposition of one class of albumins only, namely, the nucleins.[1] It appears, moreover, that the mother-substance of uric acid is confined to the true nucleins, viz., to those containing a nucleinic acid radicle, while the paranucleins, in which this is lacking, are without effect upon the elimination of uric acid. On decomposition the nucleins give rise to the appearance of the *xanthin, alloxuric,* or *purin bases,* which on oxidation are transformed to uric acid. According to Emil Fischer,[2] the xanthins are derived from an hypothetical compound which he terms *purin,* and which he supposes to be constituted as shown in the formula



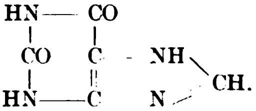
By substituting the group NH_2 for the H atom at 6, adenin thus results, and is hence also spoken of as 6-aminopurin:



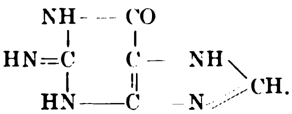
Hypoxanthin, according to this conception, would be 6-oxypurin; xanthin 2, 6-dioxypurin, and guanin 2-amino-6-oxypurin, as shown by the structural formulas:



Hypoxanthin.



Xanthin.



Guanin.

From the structural formula of purin it is also apparent that still other derivatives of this substance may exist, and as a matter of fact others are known, viz., mono-methylxanthin or heteroxanthin, di-methylxanthin or paraxanthin, tri-methylxanthin, the isomeric compounds of paraxanthin, viz., theophyllin and theobromin, and others. Their relation to xanthin is shown in the formulas:

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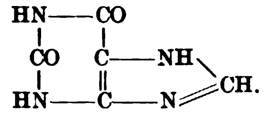
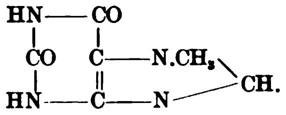
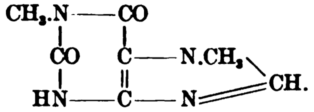
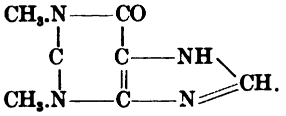
[1] C. E. Simon, Physiological Chemistry, Lea Bros. & Co.

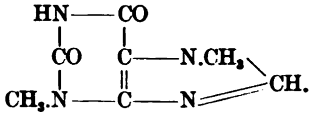
[2] Ber. d. Deutsch. chem. Ges., 1897, vol. xxx, p. 549.

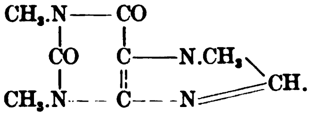

Xanthin.


Heteroxanthin.


Paraxanthin.

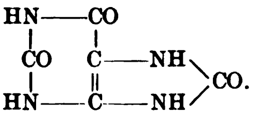

Theophyllin.


Theobromine.


Caffeine.

Two of these bodies, namely, heteroxanthin and paraxanthin, have also been found in the urine.

From these basic substances, then, which are found in the nucleinic acid radicle of the true nucleins, uric acid is supposedly derived, and there are numerous facts which go to show that this supposition is in all likelihood correct. It will thus be observed that structurally uric acid is intimately related to the bodies in question, and, like these, contains the purin radicle:


Uric acid.

It may hence be regarded as 2, 6, 8 tri-oxypurin. Uric acid and the xanthin bases, moreover, qualitatively, all yield the same decomposition products when treated with fuming hydrochloric acid or hydriotic acid under high pressure; only the quantitative relations vary, as shown in the equations:

$C_5H_5N_5 + 8H_2O = 4NH_3 + CO_2 + CH_2.NH_2.COOH + 2H.COOH.$
Adenin. Glycocoll. Formic acid.

$C_5H_4N_4O + 7H_2O = 3NH_3 + CO_2 + CH_2.NH_2.COOH + 2H.COOH.$
Hypoxanthin.

$C_5H_5N_5O + 7H_2O = 4NH_3 + 2CO_2 + CH_2.NH_2.COOH + H.COOH.$
Guanin.

$C_5H_4N_4O_2 + 6H_2O = 3NH_3 + 2CO_2 + CH_2.NH_2.COOH + H.COOH.$
Xanthin.

$C_5H_4N_4O_3 + 5H_2O = 3NH_3 + 3CO_2 + CH_2.NH_2.COOH$
Uric acid.

In accordance with this supposed origin of uric acid we find an increased elimination following the ingestion of all substances which contain purin bases either as such or in the form of true nucleins (endogenous uric acid). At the same time it must be remembered that uric acid may also result from the nucleins of the body tissues; and we find, as a matter of fact, that during starvation uric acid does not disappear from the urine (endogenous uric acid). The principal source of the uric acid under such conditions are the nucleins of the leukocytes; and, according to Horbaczewski[1] and others, this source is indeed more important than the nucleins of the food. According to this idea, the latter call forth an increased elimination of uric acid only in an indirect manner—*i. e.*, by stimulating more strongly than other food-stuffs the cell formation and cell destruction of the body. However this may be, there can be no doubt that the amount of uric acid eliminated in the urine depends, in the first instance, upon the amount of nucleins or purin bases as such which are ingested, and upon the degree of nuclear destruction which takes place in the body. Other factors, however, also enter into consideration. We thus know that the body is capable of transforming a certain amount of uric acid into urea. This fact was pointed out long ago by Frerichs and Wöhler, and has recently again been confirmed. It was found that after the ingestion of large amounts of nucleins only a certain portion of the nuclear nitrogen is eliminated as uric acid, and that this amount is extremely variable. Whether individual peculiarities have any part in determining this amount is unknown, but not improbable. Oxidation on the part of the body tissues must also be taken into consideration, and it unquestionably varies not only in different people, but also in the same individual at different times. Then again there is evidence to show that under certain conditions uric acid may be formed synthetically in the body. That this is the usual mode of formation in birds and reptiles has been shown by Minkowski,[2] who found that after extirpation of the liver in geese the greater portion of the urinary nitrogen was eliminated in the form of ammonia in combination with lactic acid. In the human being very little uric acid is in all likelihood formed in this manner under normal conditions, but the possibility of its occurrence, in disease more particularly, should not be overlooked. As uric acid, moreover, may in part at least be eliminated in the feces, it is clear that the amount which appears in the urine cannot be regarded as an accurate index of the degree of nuclear destruction or of the amount which is formed in the body tissues. That retention of uric acid can further occur in the body, which may or may not be followed by increased elimination, is likewise undoubted.

According to our present knowledge, uric acid is formed in all the organs of the body, including the bone-marrow, the muscles, the spleen, the liver, the kidneys, etc. Under pathological conditions it may also originate in the joints and tendons.

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[1] Beiträge zur Kenntniss der Bildung von Harnsäure, etc., Monatshefte für Chem., 1891, vol. xii, p. 221; and Wien. Sitzungsber, vol. c.

[2] "Ueber den Einfluss d. Leberextirpation auf den Stoffwechsel," Arch. f. exper. Path u. Pharmakol., 1886, vol. xxi, p. 41.

Under normal conditions the daily elimination of uric acid varies between 0.2 and 1.5 grams, thus constituting $\frac{1}{20}$ to $\frac{1}{120}$ part of the total urinary nitrogen. It is largely influenced by the character of the diet, the amount of exercise taken, the general health of the individual, etc. After the ingestion of large amounts of food rich in nucleins, such as thymus gland, liver, kidneys,and brain, a corresponding increase in the amount of uric acid is observed. Generally speaking, animal food causes a greater elimination of uric acid than vegetable food, and it is supposed that this difference is essentially due to the extractives of the meat.[1] Of special interest is the increase in the elimination of uric acid which is observed five hours after the ingestion of a full meal. This increase, according to Horbaczewski,[2] is associated with the disappearance of the digestive leukocytosis and consequent leukolysis.

Some observers have attached much importance to the relation existing between the elimination of uric acid and urea, and are inclined to assume the existence of a special *uric acid diathesis* when this relation continuously exceeds the usual standard of 1 to 50 or 1 to 60. This question is an extremely intricate one, and we are scarcely in a position to speak definitely of the significance of such variations. On the one hand, there can be no doubt that an unusually high uric acid coefficient may be met with in individuals who are apparently in good health, while in others, in whom larger actual amounts of uric acid are eliminated than are usual, normal or even subnormal values may be found. The entire question of the uric acid diathesis is in a chaotic condition, and it would perhaps be well to speak of such a diathesis only when a distinct increase is *continuously* observed. That numerous symptoms of a neurasthenic type are often seen when the uric acid coefficient is increased is a matter of daily observation, but it would be premature to regard this symptom as a causative factor of the disease in question.[3] Even in gout it can scarcely be said that uric acid has been proved the *materia peccans,* and our knowledge concerning the etiology of the disease is still as obscure as when Garrod[4] showed that an accumulation of uric acid occurred in the blood of such patients. Hitherto it has been supposed that the deposition of urates in the joints and periosteum of gouty patients is referable to a diminished alkalinity of the blood, and that acute paroxysms result whenever an increase in its alkalinity occurs, leading to a resorption of the urates previously deposited and a consequent flooding of the system with the material in question. As a matter of fact, a considerable diminution in its excretion is observed immediately preceding the attack, while during the paroxysm and immediately following it a corresponding increase is noted. Numerous investigations, however, have shown that distinct changes in the alkalinity of the blood do not occur in gout, and that an increase in the amount of uric acid in the blood may not only be observed in this disease, but in other diseases as well which are not associated with gouty symptoms. The conclusion is hence justifiable that the presence of uric acid in the blood *per se* cannot be offered as an explanation of the occurrence of a gouty attack.[1] Futcher,[2] who has recently observed a number of cases of gout with modern methods, states that he almost invariably found that before the onset of the acute symptoms the uric acid is below and often far below 0.4 gram. On the second or third day after the beginning of the acute symptoms the uric acid curve steadily rises, reaching 0.8 to 1.9 grams or even higher values. With the subsidence of the acute symptoms the curve gradually falls below the lower limit of the normal, and in the interval between the acute attacks the excretion may be only 0.1 to 0.2 gram daily. In one very marked chronic case Futcher found no uric acid excretion whatever on certain days during the interval. The phosphoric acid curve runs a course almost parrellel to that of the uric acid, which suggests quite strongly that even in gout the uric acid is derived from nucleins, and is not formed synthetically, as might possibly be imagined.

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[1] A Hermann, "Abhängigkeit der Harnsäureausscheidung von Nahrungs- und Genussmitteln," Deutsch. Arch. f. klin. Med., 1888, vol. xliii, p. 273. See also W. Camerer, Zeit. f. Biol., N. F., 1896, vol. xv, p. 140.

[2] Harnsäureausscheidung u. Leucocytose, Sitzungsber. d. Wiener Akad. d. Wissensch., 1891, Abth. 3. See also Löwit, Studien z. Physiol. u. Path. d. Blutes, 1892. W. Kühnau, " Das Verhältniss d. Harnsäureausscheidung zur Leucocytose," Zeit f. klin. Med., vol. xxviii, p. 534. P. F. Richter, " Ueber Harnsäureausscheidung und Leucocytose," ibid., vol. xxvii, p. 290.

[3] C. E. Simon, Amer. Jour. Med. Sci., 1899, p. 139, and N. Y. Med. Jour., 1895, p. 330.

[4] On the Nature and Treatment of Gout, 1847.

The greatest increase in the elimination of uric acid is observed in leukemia, in which the quantity may amount to over 12 grams in the twenty-four hours (case of Magnus-Levy). That the increased elimination in this disease is referable to the enormous increase in the number of the leukocytes and consequent leukolysis can scarcely be doubted. In other diseases which are associated with a high grade of leukocytosis, and especially those in which the disease terminates by crisis or hastened lysis, such as erysipelas and pneumonia, a considerable increase is likewise observed, and is referable to the same origin. This increase is especially marked immediately after crisis has occurred, but it not infrequently precedes this by several hours.

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[1] B. Laquer, Ueber die Ausscheidungsverhältnisse der Alloxurkörper. Bergmann, 1906. (Full literature.) C. von Noorden, Lehrbuch d. Pathologie d. Stoffwechsels, Berlin, 1893. W. Ebstein, "Die Natur u. Behandlung der Gicht," Verhandl. d. VIII Congr. f. inn. Med., 1889, p. 133.

[2] "The Occurrence of Gout in the United States," Jour. Amer. Med. Assoc., 1902, vol. xxxix, p. 1046.

In the other febrile diseases an absolute increase is less marked and inconstant.

In diabetes a diminished amount of uric acid is usually found. Cases may be seen, however, in which, associated with a diminution or an entire disappearance of the sugar, a most marked increase occurs, amounting in some cases to 3 grams in the twenty-four hours. To this condition the term *diabetes alternans* has been applied.

In acute articular rheumatism an increased elimination is observed so long as the temperature remains high, while with approaching convalescence the amount returns to normal, and may even fall below normal. In chronic rheumatism, on the other hand, no constant relations have been observed.

In the ordinary forms of anemia and chlorosis the amount of uric acid is quite constantly diminished, as also in chronic interstitial nephritis, chronic lead poisoning, progressive muscular atrophy, and pseudohypertrophic paralysis.

According to Krainsky, Haig[1] and Caro,[2] a decrease in the output of uric acid precedes the epileptic attack, and is subsequently followed by a rise to the same degree. Haig also noticed this in connection with attacks of migraine.

Rather low amounts are reported by Edsall in a case of purpura hemorrhagica.

Of special interest is the observation by Edsall that in those cases of chronic leukemia in which there is a response to *x*-ray treatment uric acid and purin bases are at once markedly increased.

Properties of Uric Acid.—The close relation existing between uric acid and the xanthin bases has been already considered. By oxidation uric acid is transformed into urea or into substituted ureas, such as allantoin and alloxan, which latter in turn is closely related to parabanic acid or oxalyl-urea and barbituric acid or malonyl urea.

Pure uric acid forms a white, crystalline powder which is almost insoluble in cold water (1 to 40,000), with difficulty soluble in boiling water (1 to 1800), and insoluble in alcohol and ether. In concentrated sulphuric acid it dissolves with ease, but is precipitated upon dilution with water. In aqueous solutions of the alkaline carbonates and hydrates it dissolves, with the formation of neutral or acid salts, as represented by the equations:

$$C_5H_4N_4O_3 + Na_2CO_3 = C_5H_3NaN_4O_3 + NaHCO_3.$$
$$C_5H_4N_4O_3 + 2Na_2CO_3 = C_5H_2Na_2N_4O_3 + 2NaHCO_3.$$

In freshly voided urine uric acid is said to occur as a quadriurate, viz., as a compound in which one molecule of sodium is in combination with two molecules of uric acid. The quadriurate, however, is readily decomposed with the formation of uric acid and acid urates (biurates). Its solubility in the urine depends upon the amount of water present, the reaction, and the presence of inorganic salts. When acid sodium phosphate preponderates, the biurate is precipitated, while free uric acid is thrown down when disodic phosphate only is present. Neutral urates cannot occur in the urine. The basic substances which may occur in the urine in combination with uric acid are sodium, potassium, ammonium, and possibly also calcium and magnesium. These salts may be decomposed by the addition of a sufficiently large quantity of a stronger acid, such as hydrochloric acid, when uric acid is set free. The acid salts are soluble with great difficulty, and are hence precipitated whenever the urine is markedly acid or concentrated, and also when it is exposed to a low temperature. This holds good especially for the acid ammonium compound, and upon this fact Folin's quantitative estimation of uric acid is based.

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[1] Brain, 1896, p 194.

[2] Deutsch. med. Woch., 1900, No. 19.

Pure uric acid crystallizes in transparent, colorless, rhombic plates, while that which usually separates from the urine is of a reddish-brown color and may assume a great variety of forms (Fig. 143). Of these, the so-called whetstone form is the most characteristic (see Sediments). Colorless rhombic platelets may, however, also be seen.

Of the compounds which uric acid forms with the heavy metals, the silver salt is especially important. When a solution of uric acid in ammonia is treated with an ammoniacal solution of silver nitrate (see below) the solution remains clear; but if calcium chloride, sodium chloride, or magnesia mixture is then added, a precipitate forms, which contains the uric acid in combination with silver.

Test for Uric Acid. Murexid Test.—A few crystals are dissolved by means of a few drops of concentrated nitric acid, with the application of heat, upon a porcelain plate, such as the cover of a crucible. The nitric acid is then carefully evaporated, when a yellowish-red spot will remain. Upon cooling, a drop of ammonia is placed upon this spot, when in the presence of uric acid a beautiful purplish-red color develops, owing to the formation of ammonium purpurate (murexid). If now a drop of sodium hydrate solution is added, the color changes to a reddish blue, which disappears upon heating; the reaction thus differs from the somewhat similar xanthin reaction.

Folin's Modification of Hopkins' Method.[1]—This is the most convenient method for the estimation of uric acid in the urine, and as accurate as the more complicated procedure of Ludwig-Salkowski. It is based upon the precipitation of uric acid by ammonium sulphate, as ammonium urate, the decomposition of the latter by sulphuric acid, and the estimation of the liberated uric acid by titration with potassium permanganate. To precipitate the uric acid, and also to remove the small amount of mucoid substance which is found in every urine, the following reagent is employed: 500 grams of ammonium sulphate and 5 grams of uranium acetate are dissolved in 650 c.c. of water, to which solution 60 c.c. of a 10 per cent. solution of acetic acid are further added. The resulting solution measures about 1000 c.c.; 75 c.c. of the reagent are added to 300 c.c. of urine in a flask holding 500 c.c. After standing for five minutes the mixture is filtered through two folded filters, and thus freed from the mucoid body, which is carried down with the uranium phosphate in acid solution. The filtrate is divided into two portions of 125 c.c. each, which are placed in beakers and treated with 5 c.c. of concentrated ammonia. After stirring a little the solutions are set aside until the next day. The supernatant fluid is then carefully poured off through a filter (Schleicher and Schüll, No. 597); the precipitated ammonium urate

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[1] O. Folin u. A. Shaffer, Zeit. f. physiol. Chem., vol. xxxii, p. 552.

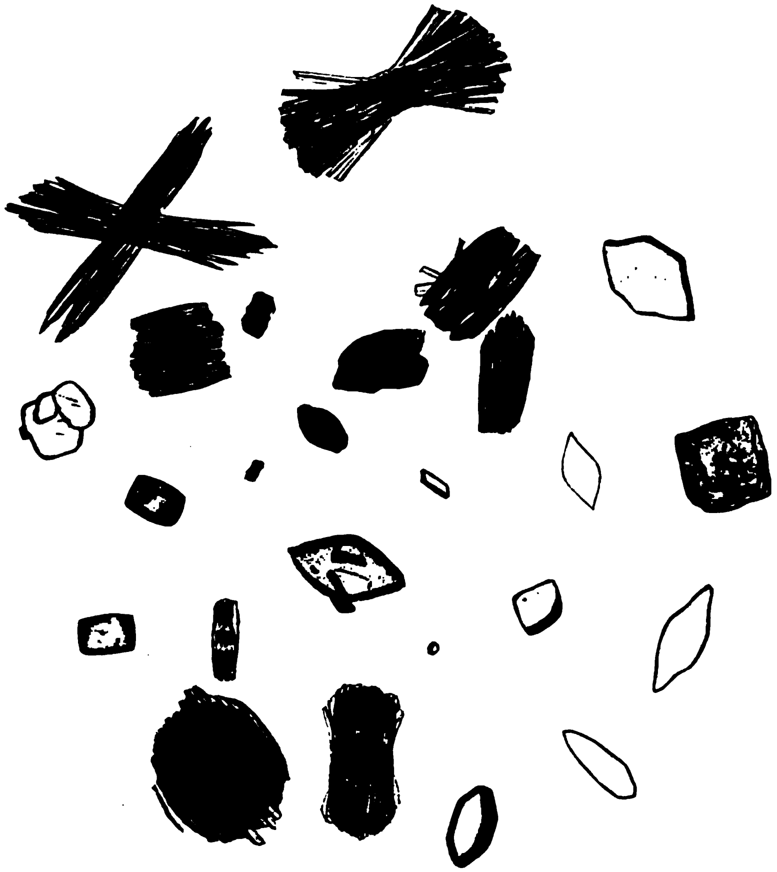

FIG. 143.—Uric acid crystals.

is collected with the aid of a small amount of a 10 per cent. solution of ammonium sulphate and washed with the same reagent. Traces of chlorides do not interfere with the subsequent titration, and the process of filtration and washing can be completed in from twenty to thirty minutes. The ammonium urate is washed into a beaker, after opening the filter, using about 100 c.c. of water; 15 c.c. of concentrated sulphuric acid are then added, and the solution is titrated at once with a one-twentieth normal solution of potassium permanganate. Toward the end of the titration Folin suggests to add the permanganate in portions of two drops at a time, until the *first* trace of a rose color is apparent throughout the entire fluid. Each cubic centimeter of the reagent corresponds to 0.00375 gram of uric acid. A final correction (of 0.003 gram for every 100 c.c. of urine employed) is necessary, owing to the slight extent to which ammonium urate is soluble.

Preparation of the One-twentieth Normal Solution of Potassium Permanganate.—As the molecular weight of potassium permanganate is 157.67, one would expect that a normal solution of the salt should contain this amount in grams dissolved in 1000 c.c. of water. But the substance generally acts in the presence of free acids, upon deoxidizing substances, by losing 5 atoms of oxygen of the 8 atoms contained in 2 molecules, as is seen in the following equation:

$$2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 10CO_2 + 8H_2O.$$

It follows that two-fifths of the molecular weight, or 63.068 grams, are the equivalent of 1 oxygen atom. But as oxygen is diatomic and the volumetric normal is calculated for monatomic values, this number must be divided by 2, and 31.534 grams of potassium permanganate should therefore be present in 1 liter of normal solution. A one-tenth normal solution would hence contain 3.1534 grams, and a one-twentieth normal solution 1.576 grams pro liter. This amount is weighed off and dissolved in 950 c.c. of water, when the solution is brought to the proper degree of dilution by titration with a one-twentieth normal solution of oxalic acid. A one-twentieth normal solution of oxalic acid contains 3.142 grams of the acid in 1000 c.c. of water. One c.c. of the one-twentieth normal solution of potassium permanganate should correspond to 1 c.c. of the oxalic acid solution. The titration is best conducted by diluting 10 c.c. of the oxalic acid solution to 100 c.c. with distilled water and adding 15 c.c. of concentrated sulphuric acid, so as to bring the temperature of the liquid to from 55° to 65° C. The potassium permanganate solution is then added drop by drop until the red color no longer disappears on stirring, but persists for at least thirty seconds.

For *Salkowski's method* of estimating uric acid see method for estimating the xanthin bases.

The Xanthin Bases.

The xanthin bases which have been found in the urine are xanthin, hypoxanthin, heteroxanthin, paraxanthin, guanin, and adenin. Conjointly they are also spoken of as the alloxur bases, or purin bases. Together with uric acid they are termed alloxur or purin bodies. Their relation to uric acid and the nucleins has already been considered. (See Uric Acid.) Unlike uric acid, they also occur as such in animal as well as vegetable tissues. The amount which appears in the urine under normal conditions is very small, constituting about 10 per cent. of the uric acid. Larger quantities may be met with in various diseases, and, generally speaking, an increase in the amount of uric acid is associated with an increase of the xanthin bases. This is, however, not invariably the case, and at times it may be observed that an increase of the uric acid is accompanied by a diminution of the xanthins, and *vice versa*. These varying relations can, of course, be readily understood if we remember that uric acid is an oxidation product of the xanthin bases, and that their ultimate origin is the same. The largest quantities of xanthin bases are found in leukemia; Magnus-Levy has reported a case with 0.321 gram.

Individually the xanthin bases are of little clinical interest. Xanthin has once been found in a urinary sediment, and has in several instances been encountered as the principal constituent of vesical calculi. Its normal quantity is said to vary between 0.02 and 0.03 gram. Larger quantities are found after a meal rich in nucleins, in leukemia, nephritis, pneumonia, etc.

Paraxanthin and heteroxanthin are present only in traces, as is apparent from the fact that Krüger and Salomon were able to obtain but 7.5 grams of heteroxanthin from 10,000 liters of urine. Both apparently are distinctly toxic.

Xanthin sediments may be recognized by means of the following test: A small amount of the material is treated with a few drops of concentrated nitric acid on a porcelain plate, and evaporated to dryness. In the presence of xanthin a yellow residue is obtained, which turns a violet red upon the addition of a few drops of sodium hydrate solution and the application of heat (Strecker's test). The reaction is common to all the xanthins and should not be confused with the murexid test.

Quantitative Estimation. Salkowski's Method.[1]—600 c.c. of urine are precipitated with 200 c.c. of magnesia mixture (composed of 1 part of crystallized magnesium sulphate, 2 parts of ammonium chloride, 4 parts of ammonium hydrate, and 8 parts of distilled water), when a 3 per cent. ammoniacal solution of silver nitrate is added to from 700 to 750 c.c. of the filtrate. The proportion should be 6 c.c.

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[1] Pflüger's Archiv, vol. lxix, p. 268.

for each 100 c.c. of urine. If the precipitated silver chloride formed in the beginning does not disappear on stirring, a little more ammonium hydrate is added. A flaky precipitate next separates out, and is allowed to settle. In order to test whether enough of the silver nitrate solution has been added, a few cubic centimeters of the supernatant fluid are acidified with nitric acid. If a distinct cloudiness, referable to silver chloride, appears, enough has been added. Otherwise the few cubic centimeters that were employed for this test are rendered alkaline again with ammonia, poured back, and treated with more silver solution until the required amount has been reached. After standing for one hour the mixture is filtered and the precipitate washed with water until all the free silver has been removed. The filter is then perforated, the precipitate washed into a flask with from 600 to 800 c.c. of water, acidified with hydrochloric acid, and decomposed with hydrogen sulphide. The excess of hydrogen sulphide is removed by heating on a water bath, when the silver sulphide is filtered off and the filtrate evaporated to dryness. The residue is treated with from 25 to 30 c.c. of dilute sulphuric acid (1 to 100). This solution is brought to the boiling point and is allowed to stand over night. The uric acid which has then separated out is filtered off, washed with a small amount of dilute sulphuric acid (not more than 50 c.c.), then with alcohol and ether, and weighed. To the resulting weight 0.0005 gram is added for each 10 c.c. of the acid filtrate, to allow for the trace of uric acid which is thus lost.

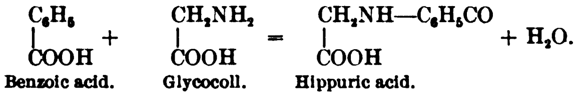
After having filtered off the uric acid the filtrate is again treated with ammonia and silver solution, and the xanthin bases thus precipitated. The precipitate is collected on a small filter, washed with water, dried, and incinerated. The ash is dissolved in nitric acid, and the silver estimated by titration with a solution of potassium sulphocyanide, using ammonioferric alum as an indicator. (See Chlorides.) The solution of potassium sulphocyanide employed in the estimation of the chlorides may be used, and is of such strength that 1 c.c. corresponds to 0.00734 gram of silver. As 1 atom of silver in a mixture of the silver compounds of guanin, xanthin, hypoxantin, etc., represents 0.277 gram of nitrogen, or 0.7381 gram of the alloxur bases, it is apparent that 1 c.c. of the potassium sulphocyanide solution will represent 0.002 gram of nitrogen and 0.00542 gram of alloxur bases. In every case an accurate record must, of course, be kept of the amount of urine and filtrate used.

The amount of alloxur bases found by Salkowski in the normal urine of twenty-four hours varied between 0.0286 and 0.0561 gram.

LITERATURE.—M. Krüger u. G. Salomon, "Die Alloxurbasen d. Harns," Zeit. f. physiol. Chem., vol. xxiv, p. 364, and vol. xxvi, 343; Deutsch. med. Woch., 1899, p. 97. Bondsynski u. Gottlieb, "Ueber Xanthinkörper im Harn des Leukämiker," Arch. f. exper. Path. u. Pharmakol., 1895, vol. xxxvi, p. 132 F. Gumprecht, "Alloxurkörper u. Leukocyten," Centralbl f. allg. Path. u. path. Anat., 1896, vol. vii, p. 820.

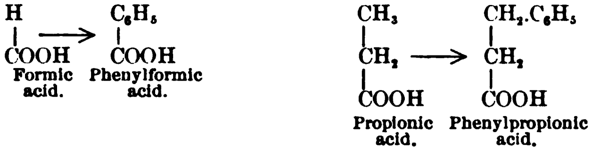
Hippuric Acid.

Hippuric acid is a constant constituent of normal urine, 0.1 to 1 gram being excreted in the twenty-four hours. That it is derived, to some extent at least, from albuminous material is proved by the fact that its elimination is not suspended during starvation nor during the administration of a purely albuminous diet. *In vitro* it may be obtained from glycocoll and benzoic acid, according to the equation



$$\begin{array}{c}C_6H_5\\|\\COOH\end{array} + \begin{array}{c}CH_2NH_2\\|\\COOH\end{array} = \begin{array}{c}CH_2NH{-}C_6H_5CO\\|\\COOH\end{array} + H_2O.$$

It has been shown that phenylpropionic acid, which differs from benzoic acid by the group C_2H_4, and which may be regarded as phenylformic acid, is produced during the process of intestinal putrefaction. The relation between the two bodies is seen from the formulas:



$$\begin{array}{c}H\\|\\COOH\end{array} \longrightarrow \begin{array}{c}C_6H_5\\|\\COOH\end{array} \qquad \begin{array}{c}CH_3\\|\\CH_2\\|\\COOH\end{array} \longrightarrow \begin{array}{c}CH_2.C_6H_5\\|\\CH_2\\|\\COOH\end{array}$$

Phenylpropionic acid is then absorbed into the blood and there, according to our present ideas, transformed into phenylformic acid or benzoic acid. When the latter comes in contact with glycocoll, which is produced during the process of pancreatic digestion, an interaction between the two substances occurs in the body, hippuric acid resulting, as shown in the above equation. This view is supported by the fact that phenylpropionic acid, just as benzoic acid, when introduced into the circulation of certain animals, reappears in the urine as hippuric acid. The final proof of the possible synthesis of hippuric acid from glycocoll and benzoic acid in the body has been furnished by Bunge and Schmiedeberg,[1] who obtained this substance, when arterialized blood containing glycocoll and sodium benzoate was allowed to pass through isolated kidneys of dogs.

Not all the hippuric acid eliminated, however, is referable to albuminous decomposition, but a considerable portion is derived from benzoic acid or its derivatives, which occur in many fruits, and are transformed into hippuric acid in the body. Among those which are particularly rich in these substances may be mentioned the red bilberry, prunes, coffee-beans, green gages, etc., and in all cases in which an increased elimination of hippuric acid is observed the possibility of this source must be taken into account.

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[1] Arch. f. exper. Path. u. Pharmakol., vol vi.

As to the seat of the synthesis there appears to be some uncertainty, as it is apparently not the same in all animals. In the dog and the frog the kidneys, according to the researches of Bunge and Schmiedeberg, must be regarded as the principal and possibly the only organs in which this process occurs. As Salomon, however, has demonstrated the presence of hippuric acid in the muscles, liver, and blood of nephrectomized rabbits, still other organs must, in the herbivora at least, be concerned in its production.

Very little is known of the pathological variations in the excretion of hippuric acid; this is principally owing to the fact that until recently suitable methods for its quantitative estimation were not available. It is an interesting fact that, in accordance with Bunge's experiments in dogs, the formation of hippuric acid appears to be suspended in cases of acute as well as chronic parenchymatous nephritis, for the benzoic acid which is then ingested reappears in the urine unchanged. In amyloid degeneration a marked diminution has likewise been demonstrated. Large quantities of hippuric acid, on the other hand, have been noted in acute febrile diseases, hepatic diseases, diabetes mellitus, chorea, etc. The data, however, are insufficient to warrant any definite conclusions.[1]

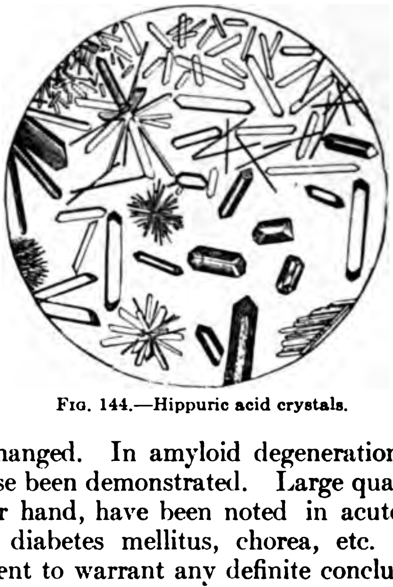

FIG. 144.—Hippuric acid crystals.

Properties of Hippuric Acid.—Hippuric acid crystallizes in long, rhombic prisms when allowed to separate from its solutions gradually, while it forms long needles if crystallization takes place rapidly and the amount is small (Fig. 144). In cold water and ether it is soluble with difficulty, while it dissolves readily in hot water, in alcohol, and in aqueous solutions of the hydrates and carbonates of the alkalies, with which it forms salts, and from which the acid may again be separated and caused to crystallize out by adding a stronger acid.

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[1] Th. Weyl u. B. von Anerep, "Ueber die Ausscheidung der Hippursäure und Benzoesäure während des Fiebers," Zeit. f. physiol. Chem., 1880, vol. iv, p. 169

When hippuric acid or one of its salts is evaporated to dryness with concentrated nitric acid and the residue is heated, the odor of bitter almonds is noticed; this is due to the formation of nitrobenzol.

When boiled with hydrochloric acid or dilute sulphuric acid hippuric acid is decomposed into glycocoll and benzoic acid. A similar decomposition is effected during the process of putrefaction, and hence no hippuric acid is found in decomposing urine, *benzoic acid* taking its place. The latter is always found in the urine together with hippuric acid, but has no clinical significance. In larger amounts it has been encountered in diabetes. It crystallizes in needles or lustrous laminæ, presenting ragged edges, which resemble plates of cholesterin. It is soluble with difficulty in cold water, but easily soluble in ether, alcohol, and solutions of the alkaline carbonates and hydrates, forming salts with the latter.

Hippuric acid in the urine occurs in combination with sodium, potassium, calcium, and magnesium.

Quantitative Estimation of Hippuric Acid.—The following method may be employed for the quantitative estimation of hippuric acid:

Principle.—Hippuric acid readily dissolves in solutions of the alkaline hydrates and carbonates, forming salts. These are decomposed by means of a stronger acid, when the hippuric acid which separates out is collected and weighed.

METHOD.—500 to 1000 c.c. of fresh urine are evaporated to a syrupy consistence on a water bath, care being taken to keep the urine neutral by the addition of sodium carbonate. The residue is extracted with cold alcohol (90 to 95 per cent.), using about half of the quantity as that of the urine employed. The mixture is then set aside for twenty-four hours. The alcoholic filtrate, which contains the salts of hippuric acid, is freed from alcohol by distillation. The remaining solution is strongly acidified with acetic acid and extracted with at least five times its volume of alcoholic ether (1 part of alcohol to 9 parts of ether). From the combined extracts the ether is distilled off and the remaining solution evaporated on a water bath. The resinous residue is boiled with water, set aside to cool, and filtered through a well-moistened filter. The hippuric acid, which is easily soluble in boiling water, is thus separated from other constituents which are soluble in alcohol and ether. The filtrate is rendered alkaline with a little milk of lime, any excess of calcium being removed by passing carbon dioxide through the mixture. This is then brought to the boiling point and filtered. Any impurities which may be present are removed by shaking with ether. The calcium salts remaining in solution are decomposed by means of an acid, when the solution is again extracted with ether. The remaining solution is evaporated to a few cubic centimeters, when the hippuric acid will separate out on standing. The crystals are dried on plates of plaster of Paris, shaken with benzol or petroleum ether to remove any benzoic acid, and finally weighed. These crystals may be shown to be hippuric acid by their microscopic appearance, their solubility in alcohol, and their behavior when evaporated with concentrated nitric acid, as indicated above.

Hofmeister's Method.—200 to 300 c.c. of urine are evaporated in a glass dish to one-third of the original volume, and treated with 4 grams of disodium phosphate, to transform the acid into its sodium salt. The mixture is evaporated to a syrupy consistence, the residue treated with burnt gypsum, dried thoroughly, and pulverized together with the dish. The powder is extracted in a Soxhlet apparatus with freshly rectified petroleum ether (boiling point 60° to 80° C.) for forty-six hours, and then for six to ten hours with pure ether (free from water and alcohol). After distilling off the ether the residue is dissolved in boiling water and decolorized with animal charcoal, the latter being subsequently thoroughly washed with boiling water; the solution and washings are evaporated to about 1 or 2 c.c. at a temperature of from 50° to 60° C., and set aside to crystallize. The crystals of hippuric acid are finally washed with a few drops of water and ether, and weighed.

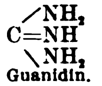
Kreatin and Kreatinin.

The antecedents of kreatin and kreatinin are unknown. Two sources of the urinary kreatinin must be recognized, viz., the muscle tissue of the body and the muscle tissue ingested as food. The tissue kreatin is possibly transformed into kreatinin and eliminated in this form, while the kreatin which has been ingested does not appear in the urine as kreatinin. Its fate is not known. Folin regards kreatinin as the essential end product of the endogenous nitrogenous katabolism, in so far at least as the muscle tissue is concerned, He has demonstrated the interesting fact that its absolute quantity on a meat-free diet is a constant quantity, which is different for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated. Its relative amount is increased when the urea nitrogen falls. On a diet rich in proteids the kreatinin nitrogen represents 3.2 to 4.5 per cent. of the total, while on one free from proteids (starch and cream) the amount may rise to 17.4 per cent. The absolute amount seems to depend to a certain extent upon the body weight. Fat or corpulent persons yield less kreatinin per unit of body weight, namely, 20 mgrms. per kilo, while lean persons yield about 25 mgrms. 1.15 to 1.6 grams may thus be regarded as average values.

The study of pathological variations in the amount of kreatinin has been greatly facilitated through the introduction of Folin's method (see below). The older data are of little importance, unless the diet of the individual has been carefully considered. A diet rich in meats, it should be borne in mind, greatly increases the amount.

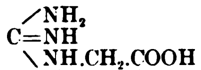
If then in patients affected with acute febrile diseases, such as pneumonia, typhoid fever, etc., a large increase is observed, the patient being at the same time upon a milk diet, an increased destruction of muscle tissue may be inferred, as a milk diet in itself, *cæteris paribus*, causes a diminished elimination. A decrease would logically be expected to occur during convalescence from such diseases. In the various forms of anemia, marasmus, chlorosis, phthisis, etc., a diminished amount is observed.[1] The same is seen in advanced cases of chronic parenchymatous nephritis, in progressive muscular atrophy, in pseudohypertrophic paralysis, and in progressive ossifying myositis.

Properties of Kreatin and Kreatinin.—Chemically, kreatin may be regarded as a methyl derivative of glucocyamin, which latter is guanidin in which 1 NH_2 group has been replaced by glycocoll. Kreatinin, on the other hand, is the methyl derivative of glucocyamidin, which differs from glucocyamin only in the absence of 1 molecule of water, so that kreatinin is kreatin minus 1 molecule of water, both being thus theoretically derivatives of guanidin. The relation between the various bodies is shown below:



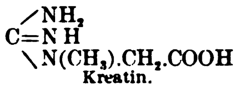
$$\begin{array}{l} \diagup NH_2 \\ C{=}NH \\ \diagdown NH_2 \end{array}$$

Guanidin.



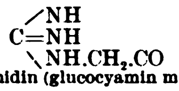
$$\begin{array}{l} \diagup NH_2 \\ C{=}NH \\ \diagdown NH.CH_2.COOH \end{array}$$

Glucocyamin.



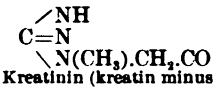
$$\begin{array}{l} \diagup NH_2 \\ C{=}NH \\ \diagdown N(CH_3).CH_2.COOH \end{array}$$

Kreatin.



$$\begin{array}{l} \diagup NH \\ C{=}NH \\ \diagdown NH.CH_2.CO \end{array}$$

Glucocyamidin (glucocyamin minus water).



$$\begin{array}{l} \diagup NH \\ C{=}N \\ \diagdown N(CH_3).CH_2.CO \end{array}$$

Kreatinin (kreatin minus water).

Kreatinin crystallizes without water of crystallization in colorless, glistening prisms. At times, when the crystals are not well developed, it also appears in the form of whetstones. It is readily soluble in hot and also quite soluble in cold water and hot alcohol; in cold alcohol and ether it dissolves with difficulty. It forms salts with acids, and double salts with some of the salts of the heavy metals. Among these may be mentioned kreatinin hydrochloride, $C_4H_7N_3O.HCl$, which is easily soluble in water and crystallizes in the form of transparent prisms or rhombic plates. Most important is the compound of kreatinin with zinc chloride, $(C_4H_7N_3O)_2.ZnCl_2$ (Fig. 145). This is produced when a watery or alcoholic solution of kreatinin is treated with zinc chloride. The crystalline form of this compound depends greatly upon the purity of the kreatinin solution. When obtained from alcoholic extracts of the urine it occurs in the form of varicose conglomerations which often adhere firmly to the walls of the vessel. If the solution of kreatinin is perfectly pure, however, it is seen in the form of fine needles grouped in rosettes or sheaves. Kreatinin-zinc chloride is soluble with much difficulty in water and insoluble in alcohol. The compound is especially important, as upon its formation and properties the quantitative estimation of kreatinin in the urine is based. Silver nitrate and mercuric chloride cause a precipitation of kreatinin, and may, therefore, also be employed for the purpose of obtaining the substance from the urine.

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[1] C. E. Simon, Physiological Chemistry, Lea Bros. & Co., 1907. Senator, Virchow's Archiv, 1876, vol. lxvii, p. 422. Neubauer u. Vogel, Harnanalyse, pt. ii.

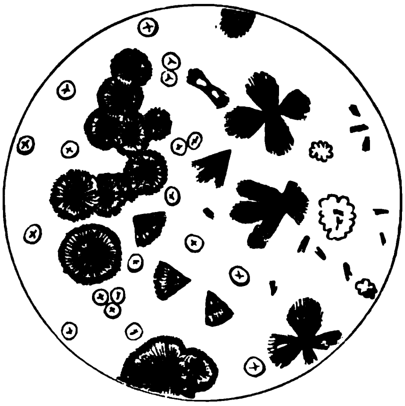

FIG. 145.—Crystals of kreatinin-zinc chloride. (Salkowski.)

Test for Kreatinin in the Urine.—A few cubic centimeters of urine are treated with a few drops of a very dilute solution of sodium nitroprusside and then drop by drop with a dilute solution of sodium hydrate. In the presence of kreatinin the urine assumes a ruby-red color, which is particularly well seen in the lower portion of the tube. This color disappears after a few minutes, and is replaced by an intense yellow, which on warming with glacial acetic acid in pure solutions turns to green, then to blue, and on standing a deposit of Prussian blue is obtained (*Weyl's test*).[1] The presence of albumin or sugar does not interfere with the reaction.

Folin's Method.[2]—This method is based on Jaffé's reaction of kreatinin with alkaline picric acid solution. The red-colored solution produced in this reaction has in proper concentration and when viewed by transmitted light exactly the same shade as a potassium bichromate solution. Half-normal potassium bichromate solution (containing 24.55 grams per liter) is therefore used as a standard for comparison. A high-grade colorimeter, by means of which the depths both of the unknown solution and of the bichromate can be adjusted to tenths of millimeters, is necessary for the comparison.[1]

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[1] Th. Weyl, Ber. d. deutsch. chem. Gesellsch., 1878, vol. xi, p. 217; and Jaffé, Zeit. f. physiol. Chem., 1886, vol. x, p. 399.

[2] The above description of the method I owe to the courtesy of Dr. Folin.

The following solutions are also necessary: The half-normal potassium bichromate solution, 10 per cent. sodic hydrate, and a saturated (1.2 per cent.) picric acid solution.

If to 10 mgrms. of chemically pure kreatinin dissolved in 10 c.c. of water in a 500 c.c. volumetric flask are added 15 c.c. of picric acid solution and 5 c.c. of sodic hydrate, the maximum color is obtained at the end of five minutes. If at the end of this time the solution be diluted to the 500 c.c. mark and at once compared with the standard bichromate solution, it will be found that 8.1 mm. of the kreatinin-picrate solution have in the colorimeter exactly the same shade and depth of color as 8 mm. of the bichromate solution.

The actual determination in urine is carried out in exactly the same way, substituting 10 c.c. of urine for the kreatinin solution. The more kreatinin that is present in the 10 c.c. of urine the deeper will, of course, be the color of the solution obtained. Supposing the colorimetric observation shows that 7.1 mm. of the urine-picrate solution are equal in color to 8 mm. of the standard, the 10 c.c. of urine would then contain $10 \times \frac{8.7}{7.1} = 11.4$ mgrms. of kreatinin.

The following precautions are to be observed in the determination:

1. Make first a preliminary colorimetric observation, using half-normal potassium bichromate solution in both cylinders of the colorimeter, adjusting first one to the 8 mm. mark. The average of three or four readings of the other cylinder should also be 8 mm., and after the first observation no two should differ by more than 0.2 mm. This preliminary observation takes only two or three minutes, and is exceedingly useful in making the eye sure of the correct point to be ascertained.

2. Exactly 8 mm. of the half-normal potassium bichromate solution must be used as the standard for comparison. 16 or 24 mm., for example, cannot be substituted on the basis of the calculation given above because the kreatinin-picrate solution absorbs light at an entirely different rate from that of the bichromate solution.

3. For the reason given in the preceding paragraph it is necessary to make each determination with a quantity of urine containing not less than 5 nor more than 15 mgrms. of kreatinin. Within these limits the determination as described is correct within 0.2 mgrm.

4. Sugar and albumin do not interfere with the determination. Acetone, diacetic acid, and hydrogen sulphide do interfere. Where

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[1] The French instrument of *Duboscq*, which can be obtained through *Eimer & Amend*, is admirably suited for the purpose.

these are present the urine should be measured into a porcelain evaporating dish and heated on a water bath with 10 c.c. of 1 per cent hydrochloric acid for about half an hour. When the dish is again cooled, the reagents are added directly into the dish, and finally rinsed into the volumetric flask after five minutes.

5. The color due to the urine is ordinarily of no appreciable consequence because of the great dilution. Urines containing bile pigments can, however, first be cleared by the addition of egg albumen and then removing this by coagulation (heat).

The whole operation can be finished in less than fifteen minutes; indeed, it should be finished at once, as the colored product obtained by the interaction of kreatinin and picric acid is not very stable.

Oxalic Acid.

The origin of oxalic acid in normal urine is twofold. The greater portion is supposedly derived from the ingested food, but there is evidence to show that a certain amount is also formed during the metabolism of the body tissues, as the elimination of oxalic acid does not cease during starvation. The carbohydrates and fats probably do not play a part in this connection; and, according to Salkowski, the albumins also do not enter into consideration *per se*. He rather inclines to the view that the nucleins represent the antecedent of the oxalic acid, and as a matter of fact uric acid, which, as we have seen, is itself derived from the nucleinic bases, can be readily oxidized to oxalic acid, with the intermediary formation of parabamic acid and *oxaluric acid*. The latter has been repeatedly demonstrated in the urine, and it is conceivable that the same process may occur in the animal body. But even supposing that the oxaluric acid which is obtained from the urine is formed artificially during the lengthy process of analysis, and that the substance did not exist preformed, there is no reason for the assumption that uric acid may not be the normal antecedent of the oxalic acid. For Salkowski has demonstrated conclusively that on oxidation with ferric chloride in aqueous solution uric acid yields oxalic acid and urea directly.

The matter, however, is not quite so simple as it appears, and an increased elimination of oxalic acid by no means always occurs when the output of uric acid is increased. After the ingestion of fairly large amounts of thymus, for example, the usual increase of uric acid is not accompanied by a corresponding increase in the amount of oxalic acid, and in those cases in which it does occur we are as yet unable to exclude the large amount of connective tissue as the source of the oxalic acid. Connective tissue and gelatin have, as a matter of fact, been shown to increase the amount of oxalic acid when given in large amounts. With pure nuclein no effect has been observed, and it can be shown that in those experiments in which this was used by mouth an absorption from the intestinal tract had manifestly not occurred (Mohr and Salomon).[1]

Under pathological conditions oxalic acid may also be formed in the digestive tract from the ingested carbohydrates, as a result of a peculiar fermentative process. This has been well shown by Helen Baldwin in Herter's laboratory. In some of these cases no free hydrochloric acid could be demonstrated in the gastric contents, and it was observed that inoculation of a digestive mixture, which was originally free from oxalic acid, resulted in its appearance if a few drops of such stomach contents were added. In dogs prolonged feeding with excessive quantities of glucose together with meat was seen to lead eventually to a state of oxaluria, which was associated with a mucous gastritis and the absence of free hydrochloric acid. Oxalic acid could then also be demonstrated in the stomach contents.

Very curiously the ingestion of quite small and non-toxic amounts of oxalic acid is followed by a fairly intense indicanuria. It does not seem likely to me, however, that as Harnack and v. d. Leyen suggest, the indicanuria is here referable to a toxic action upon the tissue albumins, and I am personally inclined to explain the phenomenon upon the basis of increased intestinal putrefaction. (See Indicanuria.)

The amount of oxalic acid which is normally eliminated in the twenty-four hours fluctuates with the amount ingested, and varies from a few milligrams to 2 or 3 centigrams, being usually less than 10 milligrams (Baldwin). It is influenced by the character of the diet. The ingestion of oxalates by the mouth is followed by their partial elimination only in urine and feces, so that we may conclude that to a certain extent oxalic acid is decomposed during its passage through the animal body; possibly this may occur in the intestinal canal as the result of bacterial action.

Foods rich in oxalic acid are spinach, tomatoes, carrots, celery, string-beans, rhubarb, potato, dried figs, plums, strawberries, cocoa, tea, coffee, and pepper. Foods which contain little or no oxalic acid, on the other hand, are meat, milk, eggs, butter, cornmeal, rice, peas, asparagus, cucumbers, mushrooms, onions, lettuce, cauliflower, pears, peaches, grapes, melons, and wheat, rye, and oat flour.

Before drawing conclusions as to the existence of abnormal oxaluria it is hence imperative to eliminate the possibility of an increased ingestion, by placing the patient upon a diet which contains little or no oxalic acid.

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[1] Deutsch. Arch. f. klin. Med., 1901, vol. lxx, p. 486. Lommel, ibid., vol. lxiii, p 599

An increased elimination is notably observed in association with various dyspeptic and nervous manifestations, and constitutes the condition commonly spoken of as the *oxalic acid diathesis*, or as *idiopathic oxaluria*. Its existence as a definite pathological picture is, however, denied by most modern clinicians. Nevertheless it must be admitted that there is a certain type of neurasthenia in which, generally in association with hyperchlorhydria, an increased elimination of oxalic acid takes place, and in which a copious deposit of calcium oxalate crystals is frequently observed. From the mere fact of the occurrence of such deposits, of course, no inference is, as a rule, to be drawn regarding the actual elimination, but its frequent occurrence is in itself of importance, as in such cases a similar separation from the urine may already occur within the urinary passages, and not uncommonly in the pelvis of the kidneys. Not infrequently oxaluria of this type is associated with an increased elimination of uric acid and a mild grade of albuminuria, as has been shown by Senator, von Noorden, Da Costa, myself, and others. Whether or not the oxaluria in these cases can be explained upon the basis of abnormal fermentations in the gastro-intestinal tract, as is suggested by the observations of Baldwin, remains to be seen. In some this may be the case, but in others I am inclined to associate the oxaluria with the coexistent lithuria.

Very interesting is the apparently vicarious oxaluria which is at times observed in diabetes. Fürbringer has reported a case of diabetes in which the elimination of oxalic acid was described as "enormous," and in which oxalic acid could also be demonstrated in the sputum (oxaloptysis). Rausch has recorded a case of mild diabetes, associated with hepatic cirrhosis, in which 1.2 grams were excreted in twenty-four hours. In most cases of diabetes, on the other hand, an increased oxaluria cannot be demonstrated.

In cases of obesity Kisch found no abnormal degree of oxaluria.

In association with jaundice increased oxaluria has been repeatedly observed, and is probably referable to biliary stasis and consequent cholemia, as Salkowski has demonstrated that the bile contains oxalic acid. In pneumonia and leukemia, in both of which we find as a rule a greatly increased elimination of uric acid, the oxalic acid is not always increased, and sometimes indeed quite low in comparison with the amount of uric acid.

Properties of Oxalic Acid.—Oxalic acid occurs in the urine as calcium oxalate, CaC_2O_4, and is held in solution by the diacid sodium phosphate. It can, hence, be precipitated by diminishing the acidity of the urine by the addition of a little ammonia or by allowing it to stand exposed to the air. When allowed to crystallize out slowly, calcium oxalate occurs in the form of well-defined, strongly refractive octahedra, in which the principal axis of the crystals is placed at right angles to the plane of the microscopic slide (Fig. 146). These are very characteristic. Other forms, however, are also quite commonly observed, such as single and double dumb-bells, spheroids and prisms, etc. They are insoluble in ammonia and alcohol, almost insoluble in hot and cold water, and very slightly soluble in acetic acid, but dissolve with ease in the mineral acids.

When strongly heated, the salt is decomposed into calcium oxide, carbon dioxide, and carbon monoxide.

Tests for Oxalic Acid.—For the detection of calcium oxalate it is frequently only necessary to examine the sediment of the urine after standing for twenty-four to forty-eight hours. No oxalate crystals, however, may be found even when an abnormally large amount can be demonstrated by chemical methods. In such cases it is usually possible to bring about the crystallization of the salt by carefully neutralizing the urine with a little ammonia. Should this procedure not lead to the desired end, it is best to treat the urine with one-third its volume of 95 per cent. alcohol. The mixture is set aside for twenty-four to forty-eight hours, when the sediment is centrifugalized and examined with the microscope. This method, Baldwin states, represents a more delicate test for oxalic acid than the complicated methods of quantitative analysis which are available.

Quantitative Estimation.—Heretofore the old method of Neubauer has been in general use, but it is at best unsatisfactory. It has been replaced by the methods of Dunlop and Salkowski.

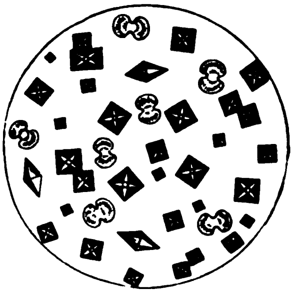

FIG. 146.—Calcium oxalate crystals.

Dunlop's Method (slightly modified by Baldwin).—In this case the calcium oxalate is precipitated from an acid solution by means of alcohol, instead of from an alkaline solution by calcium chloride. The urine is thymolized, and, if alkaline, acidified with a trace of acetic acid; 500 c.c. of a well-mixed specimen of the collected urine of twenty-four hours are treated with 150 c.c. of over 90 per cent. alcohol, to precipitate the calcium oxalate. The mixture is set aside for forty-eight hours. It is then filtered, care being taken to ensure the entire removal of the crystals from the beaker. The sediment is thoroughly washed with hot and cold water, and finally with dilute acetic acid (1 per cent. solution). The filter is placed in a small beaker and soaked in a small amount of dilute hydrochloric acid. It is then washed with hot water until the washings no longer give an acid reaction. The acid solution and washings are filtered, and the filtrate evaporated to about 20 c.c. This is treated with a very small amount of a solution of calcium chloride, to ensure the presence of an excess of calcium. The solution is neutralized with ammonia, slightly acidified with acetic acid, and treated with strong alcohol, so that the mixture contains 50 per cent. After forty-eight hours the sediment is collected on a filter free from mineral ash, and is washed with cold water and dilute acetic acid until free from chlorides. The filter with its contents is then incinerated, first over a Bunsen burner, and afterward for five minutes in a blow-pipe flame. On cooling over sulphuric acid the ash is weighed; the result multiplied by 1.6 represents the amount of oxalic acid in the volume of urine examined.

Salkowski's Method.—In the case of human urine of moderate concentration 500 c.c. of the non-filtered urine are evaporated to about one-third. On cooling, the liquid is acidified with 20 c.c. of hydrochloric acid (sp. gr. 1.12), and extracted three times with new portions of 200 c.c. each of a mixture of 9 to 10 volumes of ether and 1 volume of alcohol. The ethereal extracts, which contain the liberated oxalic acid are carefully separated from the urine and filtered through a dry filter. The ether is distilled off; the remaining alcoholic solution, which still contains a little ether, is placed in a deep evaporating dish, diluted with 10 to 15 c.c. of water, and evaporated on a water bath. The resulting milky fluid is concentrated, more water being added if necessary, until it becomes clear and a gummy material separates out. On cooling, the liquid, which should measure about 20 c.c., is passed through a small filter. This is washed once or twice with a *little* water, when filtrate and washings are rendered slightly alkaline with ammonia, treated with 1 to 2 c.c. of a 10 per cent. solution of calcium chloride, and acidified with dilute acetic acid. The reaction should be distinctly acid, but an excess should be avoided. An indication that a sufficient amount has been added is afforded by the dissolution of the precipitate of phosphates, which occurs after the addition of the calcium chloride solution. After standing for twenty-four hours, or, still better, forty-eight hours, the calcium oxalate that has separated out is collected on a filter free from ash, washed with hot and cold water, dried, and incinerated as usual (see above). The resulting weight multiplied by 1.6 indicates the corresponding amount of oxalic acid in grams.

LITERATURE.--P. Fürbringer, "Zur Oxalsäureausscheidung durch d. Harn." Deutsch. Arch. f. klin. Med., 1876, vol. xviii, p. 143. J. C. Dunlop, "The Elimination of Oxalic Acid in the Urine," etc., Jour. Path. and Bact., 1896 (an historical review of the subject of oxaluria is here also given). H. Baldwin, "An Experimental Study of Oxaluria," Jour. Exper. Med., vol. v, p. 27. E. Salkowski, Berlin. klin. Woch., 1900, p. 434; and Zeit. f. physiol. Chem., vol. xxix, p. 437. E. Harnack, "Ueber Indicanurie in Folge von Oxalsäurewirkung," Zeit. f. physiol. Chem., 1900, vol. xxix, p. 205.

Albumins.

The albumins which may be met with in the urine are serum albumin, serum globulin, albumoses (peptones), the albumin of

Bence Jones, hemoglobin, nucleo-albumin, fibrin, histon, and nucleohiston. Of these, serum albumin is the most important from a clinical standpoint.

Serum Albumin.—The question whether or not serum albumin occurs normally in the urine—*i. e.*, under strictly physiological conditions—has been much disputed. It is claimed by some that traces may be temporarily met with in apparently healthy individuals after severe muscular exercise, cold baths, mental labor, severe emotions, during menstruation, digestion, etc. This so-called *physiological albuminuria* mostly occurs in young adults, and is usually, if not always, of brief duration. The urine, it is claimed, is otherwise normal—*i. e.*, of normal amount, appearance, specific gravity, and composition, and free from abnormal morphological constituents, such as casts, red corpuscles, leukocytes, and epithelial cells.[1] However, Darling[2] has shown that severe muscular exercise may produce a urinary picture which, even though temporary, closely simulates what is seen in acute nephritis. He reports 0.9 per cent. of albumin in a member of a Harvard four-oared crew after a two-mile race, and amounts varying from 0.25 to 0.5 per cent. in five others under similar conditions. The sediment at the same time contained large numbers of hyaline and finely granular casts, many with renal cells and red blood corpuscles adherent. In many of the sediments there were also numerous red cells as such and an excess of leukocytes.

The existence of a physiological albuminuria, on the other hand, is denied, and the occurrence of serum albumin at least regarded as pathological in every case. I have never been able to convince myself of the occurrence of serum albumin in the urine under strictly physiological conditions, and am hardly prepared to regard severe muscular and mental labor, severe mental emotions, cold baths, etc., as physiological stimuli. The albuminuria, so often observed during the first days of life, at which time sediments of uric acid and urates, mucus, epithelial cells from the different portions of the urinary tract, and even casts may also be seen—*i. e.*, constituents which in adults would rightly be regarded as abnormal—has also been brought forward in support of the theory of a physiological albuminuria. There can be no doubt, however, that this form of albuminuria is referable to the profound changes that take place in the circulatory system after birth, and to some extent perhaps also to the well-known uric acid infarctions so frequently seen in the kidneys of the newly born, so that it would probably be better and more in accord with the teachings of pathology to regard this form of albuminuria also as abnormal.[3]

The more closely the subject of the so-called physiological albuminuria is studied, the more improbable does its physiological nature appear, and a more detailed study of the metabolic processes, it may be confidently asserted, will ultimately lead to the conclusion that *the presence of albumin in every case is a pathological phenomenon.*

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[1] C. E. Simon, "Functional Albuminuria," N. Y. Med. Jour., 1895, p. 330.

[2] Boston Med. and Surg. Jour., September 7, 1899, p. 231.

[3] L. Landi, L'albuminuria nel parto, Morgagni, 1890, vol. xxxii

The association of an increased elimination of urea and uric acid with albuminuria in apparently healthy individuals was noted many years ago, but received comparatively little attention.[1] Personal observations have led me to look upon this form of albuminuria as of common occurrence, and while in almost every case the albumin can be caused to disappear from the urine by proper diet and exercise, there can be no doubt that, if neglected, granular atrophy may ultimately result.

An albuminuria may at times be observed in anemic children and adolescents, and particularly in masturbating boys of the mouth-breathing type, but can hardly be regarded as physiological. The same may be said of the albuminuria of pregnancy and parturition.

As regards the action of cold baths, Rem-Picci[2] reports that albuminuria may be considered a constant phenomenon after cold baths, but that different subjects react differently under the same conditions. Those which show albuminuria more readily are, as a rule, the less robust and thinner individuals, such as are most sensitive to cold. The limits of temperature necessary to produce the phenomenon are from 12° to 13° C., when the immersion is not longer than three minutes. If the temperature be from 15° to 20° C., the albumin appears only after fifteen minutes' immersion. Above this temperature albuminuria does not occur, even if the bath lasts much longer. The colder the bath, the more rapid the appearance of albumin. The degree of albuminuria is always slight, and even in the more marked cases rarely exceeds 0.25 pro mille. The sediment, according to Rem-Picci, occasionally shows a few hyaline casts, and often crystals of calcium oxalate.

The course which may be taken by these various forms of what should be termed *functional* albuminuria, in which the amount of albumin rarely exceeds 0.1 per cent., is very interesting. The elimination of albumin may thus be quite *transitory* on the one hand, as when following severe muscular exercise, cold baths, and the like. It may, however, also last for several days, or even weeks, and be followed by a disappearance of the albumin for a variable length of time, and again by its reappearance and continuance for days and weeks. The term *intermittent albuminuria*[3] has been applied to this latter type. At times the albuminuria may follow a definite course, disappearing and reappearing with such regularity that it has not improperly been styled *cyclic albuminuria.*[1] In this form the albumin generally disappears from the urine during the night or during prolonged rest in bed, and reappears during the day, the erect posture apparently favoring its reappearance; the term *postural* or *orthostatic albuminuria* has hence also been suggested for this form. Oswald, who made a careful study of cyclic albuminuria in Riegel's clinic, regards its occurrence as distinctly pathological, and as indicating the existence of nephritis. Remembering the importance of the subject, it may not be out of place to enumerate the reasons which led Oswald[2] to this conclusion:

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[1] Da Costa, "The Albuminuria and Bright's Disease of Uric Acid and Oxalic Acid," Amer. Jour. Med. Sci., 1895.

[2] "On Albuminuria after Cold Baths," Il Policlinico, 1901, vol. viii, p. 389.

[3] Bull, Berlin. klin. Woch., 1886, vol. xxiii, p. 717. Marcau, Rev. de méd., 1886, vol. vi, p. 855. Klemperer, Zeit. f. klin. Med., 1887, vol. xii, p. 168.

1. The patients generally come to the physician complaining of certain definite symptoms which are similar to those noted in cases of true nephritis. At times, however, no complaints are made, because the patients have reasons for concealing them (as in examinations for life insurance), or because they are temporarily absent.
2. The subjective complaints, as well as the anemia so frequently observed in such cases, generally disappear, together with the albumin, under suitable treatment, and reappear when the anemia again becomes marked.
3. In many a history of an antecedent nephritis the result of scarlatina or diphtheria may be obtained, as in 3 cases of Heubner, in 14 cases out of 20 described by Johnson, etc. In some also a direct transition from an acute nephritis to the cyclic form of albuminuria has been noted. Where this was not possible the history of an acute infectious disease or an angina that had been overlooked in the clinical history must be regarded as a possible cause.
4. The absence of morphological elements, especially tube casts, does not exclude a nephritis. A large number of cases, moreover, have recently been observed in which casts were repeatedly found.
5. A cyclic albuminuria may be observed in many cases of chronic nephritis.
6. Marked organic abnormalities (such as heart lesions) need not be demonstrable, as they may be absent for a long period of time or may be unrecognizable.

According to the researches of Erlanger and Hooker[3] orthostatic albuminuria is dependent upon a lowering of the pulse pressure (being the difference between the minimum and the maximum blood pressure), which constantly occurs when the individual changes from the recumbent to the erect posture. In the true form of orthostatic albuminuria the albumin present is serum albumin. Casts are absent.[4]

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[1] A. Keller, Beiträge z. Kenntniss d. cyklischen Albuminurie, Diss., Breslau, 1896.

[2] "Cyklische Albuminurie u. Nephritis," Zeit. f. klin. Med., vol. xxvii, p. 73.

[3] Johns Hopkins Hosp. Reports, 1904, p. 346.

[4] Teissier, Rev. de méd., April 10, 1905, p. 233.

It may be safely asserted that a transitory, intermittent, and cyclic albuminuria is not infrequently observed in apparently healthy individuals, but that the facts so far brought forward do not warrant the assumption that such forms of albuminuria are physiological.[1] The occurrence of such albuminuria unquestionably demonstrates a certain insufficiency of the renal epithelium, and I am much in favor, as Martius has proposed, of discarding the term *physiological* albuminuria altogether, and to speak of these various forms collectively as *constitutional* albuminuria.

It would lead too far to enter into a detailed consideration of the various causes that have from time to time been suggested as an explanation of the fact that albumin does not occur in the urine under normal conditions. There can be no doubt, however, that the integrity of the epithelial lining of the glomeruli and the convoluted tubules must be regarded as the principal factor which prevents the albumin of the blood from passing into the urine. When the readiness with which the glandular structures of the kidney respond to any abnormal stimulation is considered, it is easily understood how an albuminuria may be produced in many different ways. Aside from acute and chronic inflammatory processes in the widest sense of the word, an albuminuria may be the result of circulatory disturbances in the kidneys of whatever kind—*i. e.*, the result of anemia as well as of hyperemia. In many and perhaps the majority of cases of what Bamberger[2] terms *hematogenous albuminuria*, we have direct evidence of the existence of circulatory disturbances, as in cases of uncompensated valvular lesion, weak heart, emphysema, hepatic cirrhosis, etc. In other cases, however, the existence of such disturbances can only be surmised, and the question, whether or not the albuminuria observed in the various infectious diseases, for example, is referable to circulatory abnormalities or to a direct irritative action of microbic poisons upon the renal parenchyma, must still remain open.

From personal studies in connection with the functional albuminuria of Da Costa, it seems not unlikely that in many cases in which obscure circulatory disturbances are supposed to exist and are held responsible for an existing albuminuria, this is referable rather to the strain thrown upon the kidneys by the continued elimination of abnormally large quantities of organic material, the quantity of water being at the same time proportionately small.

If it is remembered, furthermore, that injuries affecting certain portions of the brain are followed by albuminuria, and that this may be artificially produced by a *piqure*, analogous to the glucosuric

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[1] v. Noorden, Deutsch. Arch. f. klin. Med., vol. xxxviii, pp. 3 and 205. Leube, Zeit. f. klin. Med., 1887, vol. xiii, p. 1. Winternitz, Zeit. f. physiol. Chem., 1891, vol. xv, p. 189. C. E. Simon, loc. cit.

[2] Wien. med. Woch., 1881, pp. 145 and 177.

iquure of C. Bernard, still another factor is given which may possibly nter into the causation of albuminuria.

Obstruction to the outflow of urine from the kidneys has also een experimentally shown to lead to albuminuria, an observation ith which clinical experience is in perfect accord.

In patients actually in labor albuminuria is common, and supposedly due to increased blood pressure in the kidneys caused by uterine contractions and the general disturbance of the circulation. The relative frequency of its occurrence is a matter of dispute, however, and widely differing statements are made by different observers, ranging from 15 to 20 per cent. (Petit, Winckel) to 99 and 100 per cent. (Trautenroth, Pajikull).

As regards the occurrence of albuminuria in pregnancy the results of different observers likewise differ, viz., from 1 to 50 per cent. In the last months of pregnancy Zangemeister[1] found albumin in 10 per cent. of the cases examined, and if repeated examinations were made positive results were obtained persistently during the last three months in 40 per cent. The albuminuria is supposedly referable to some metabolic disturbance and impaired excretion by the kidneys.

Finally, an abnormal composition of the blood may at times cause the albuminuria.

In passing on to a more detailed study of the various pathological conditions in which an elimination of albumin may be noted, an attempt will be made to classify the various forms of albuminuria in accordance with the more general considerations set forth above. It should be remembered, however, as already indicated, that it may be very difficult, if not impossible, to assign one single cause to a given clinical case, as several factors may at the same time be operative in the production of the albuminuria.

1. Functional Albuminuria.—Under this heading may be comprised the various forms of "physiological" albuminuria, which have already been considered.

2. The Albuminuria associated with Organic Diseases of the Kidneys, viz., acute and chronic nephritis, renal arteriosclerosis, amyloid degeneration of the kidneys.[2]

In acute nephritis, albuminuria, usually of great intensity, is a constant and most important symptom. The amount eliminated is generally proportionate to the intensity of the disease, but varies within fairly wide limits, generally from 0.3 to 1 per cent., corresponding to a daily excretion of from 5 to 8 grams. Much larger quantities, it is true, are at times excreted, but it may be definitely stated that the daily loss of albumin seldom exceeds 20 grams.

In chronic parenchymatous nephritis the elimination of albumin is likewise constant, and the amount excreted in severe cases may even exceed that observed in the acute form. An elimination of from 15 to 30 grams, viz., 1.5 to 3 per cent. by weight, is frequently observed.

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[1] Arch. f. Gyn., 1902, vol. lxvi, Heft 2.

[2] Senator, loc. cit.

In the ordinary form of chronic interstitial nephritis the elimination of albumin is, as a general rule, slight, and rarely amounts to more than 2 to 5 grams pro die. At the same time it is not unusual to meet with an apparent absence of albumin if the more common tests (see below) are employed. If it is remembered that very often the diagnosis of the disease is dependent upon the demonstration of the presence or absence of albumin, the necessity of *frequent* examinations and the employment of more delicate tests, particularly of the trichloracetic acid test, as well as of a microscopic examination, is at once apparent. This is even of greater importance in the renal arteriosclerosis of Senator, in which albumin by the ordinary tests is probably not demonstrable in the majority of cases, and in which even the trichloracetic acid test *may* not be of service, and casts be absent.

Amyloid degeneration of the kidneys, in the absence of inflammatory processes, is accompanied by a condition of the urine closely resembling that observed in the ordinary form of chronic interstitial nephritis. A total absence of albumin, however, is less frequently noted, while an amount varying between 1 and 2 per cent. is not uncommon. It will be shown later on that in this condition considerable amounts of serum globulin are excreted in addition to the serum albumin; larger amounts, in fact, than are generally observed in this form of chronic renal disease; so that Senator suggests that such a relation, in the absence of an acute nephritis, or an acute exacerbation of a chronic nephritis, may be of a certain diagnostic value.

3. FEBRILE ALBUMINURIA.[1]—That albuminuria may occur in almost any one of the various febrile diseases is a well-known fact, but it is important to remember that, while such an albuminuria *may* at times be referable to a true nephritis developing in the course of or during convalescence from an acute febrile disease, such is the exception, and not the rule. Under this heading, only that form will be considered which is not associated with distinct changes affecting the renal parenchyma, and which generally appears during the height of the disease only, and disappears with a return of the temperature to normal. As has been mentioned, it is often difficult, if not impossible, to assign a definite cause for an albuminuria of this character, and in all probability several factors are in operation at the same time. In the beginning of the disease, when the blood pressure, as a rule, is increased, the albuminuria may be

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[1] Leyden, Zeit. f. klin. Med., 1881, vol. iii, p. 161. H. Lorenz, Wien. klin. Woch., 1888, vol. i, p. 119.

referable to an ischemia of the kidneys, as the increased pressure in fever, according to Cohnheim and Mendelson, is largely referable to spasm of the arterioles. Later on, or in the beginning of cases in which especially severe intoxication exists, the blood pressure may be subnormal, and the albuminuria be due to this cause—*i. e.*, a hyperemic condition of the kidneys. As a matter of fact, it has been experimentally demonstrated that both anemia and hyperemia of the kidney structure may lead to albuminuria. On the other hand, it is not unlikely that the strain thrown upon the kidneys by an excessive elimination of organic material, in the absence of a correspondingly large quantity of water, may produce albuminuria. I have repeatedly seen the functional albuminuria of the type described by Da Costa disappear during the administration of a diet relatively poor in nitrogen, while an increased diuresis was at the same time effected by the consumption of large amounts of water.

In those grave cases of typhoid fever, furthermore, which are characterized by high fever and pronounced nervous symptoms, it would appear quite likely that the albuminuria, which in these cases is particularly marked, is referable to a direct influence upon the central nervous system, and in some cases, at least, also dependent upon an irritant action upon the renal epithelium on the part of the microbic poisons circulating in the blood. The character of the albuminuria will largely depend upon the intensity of the intoxication; in other words, upon the amount of bacterial poison present at any one time in the blood.

Notwithstanding statements to the contrary, albuminuria may be regarded as a constant symptom of typhoid fever, as has been definitely demonstrated by Gubler and Robin. It is difficult to say why other observers have found albumin in only a comparatively small percentage of cases, but it is not unlikely that this is owing to a lack of uniformity in methods, it being presupposed also that questions of this kind can only be decided by *daily* examinations. According to Robin, the trace of albumin which is at times observed during the first week of the disease is an albumose, while later on serum albumin is constantly found; the amount increases with the intensity of the morbid process, and the highest figures are reached in fatal cases. The more severe the disease, the earlier does albumin appear in the urine, it being remembered, however, that reference is had only to those cases in which distinct renal changes are not demonstrable. Toward the termination of the fastigium the amount of albumin generally undergoes a certain diminution, and may even disappear entirely. This diminution, however, is only temporary, and in severe cases the albumin again increases in amount during the period of great variations in the temperature. In light cases an increased elimination also takes place at this stage, but is soon followed by a decrease, after which traces only can be demonstrated.

In some cases it disappears entirely, but it is rare, according to Robin, to meet with cases in which a trace at least does not reappear during convalescence.

In light cases the albuminuria rarely persists longer than the fifth or eighth day of convalescence, and Robin even goes so far as to say that a relapse may be anticipated if the albuminuria does not disappear at that time. A limited number of personal observations have borne out the correctness of this view. In severe cases, on the other hand, the albumin persists for a variable length of time, and rarely disappears before the tenth day of convalescence. At times an increase is seen during convalescence when traces only have previously been observed. It is this form which the French generally speak of as *colliquative albuminuria.* While this is principally observed in typhoid fever, it is not unusual to meet with it during convalescence from various other acute diseases. Care must be taken not to confound the albuminuria so frequently seen during convalescence from typhoid fever, referable to a pyelitis, with the form just described.

From the following summary, constructed from data given in Robin's[1] monograph on the urine of typhoid fever and other acute infectious diseases which may be associated with a typhoid condition, an idea may be formed of the occurrence of albuminuria, as well as of its degree of intensity in these diseases:

Acute miliary tuberculosis: albumin is much less frequent than in typhoid fever; when present, it is rarely found in the abundance so characteristic of the fatal cases of the latter disease.

Pneumonia: albumin is as uniformly present as in typhoid fever, and at times very abundant.

Grippe: albumin is infrequent; present in about 20 per cent. of the cases, and only in traces.

Herpetic fever: albumin never present in large amounts.

Embarras gastrique: albumin rarely present.

Adynamic enteritis of adults: albumin almost always present, but usually only in traces.

Cerebrospinal meningitis: albumin in fairly large amounts.

Malignant endocarditis: albumin very abundant in about 14 per cent., evident in 44 per cent., and traces in 42 per cent. of all cases.

Acute articular rheumatism: albumin present in about 40 per cent.

Rubeola: albumin usually absent in light cases, but present in the more severe and complicated forms.

Intermittent fever: albumin variable.

In a series of 799 cases of pneumonia reported from the Boston City Hospital,[2] albumin was found in 624—*i. e.*, in 78 per cent. It was noted that the death rate bore a direct ratio to the amount of albumin in the urine.

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[1] Urologie clinique de la fièvre typhoide, Paris, 1877.

[2] Sears and Larrabee, Med. and Surg. Rep. of the Boston City Hospital, 12th Series, Dec., 1901.

In smallpox a trace of albumin is practically constant. Somewhat larger amounts are found in about 30 per cent. of all cases. The albuminuria is most marked during the eruptive stage and then rapidly diminishes in intensity. More rarely it reaches its maximum during the suppurative fever stage, or during convalescence.[1]

As the result of the examination of a large number of cases of plague Corthorn[2] arrived at the conclusion that no albumin is found in only 14 per cent. of all cases. In cases ending in recovery the albuminuria never occurred later than the fourth day.

In conclusion, it may be said that practically every acute febrile disease, even simple follicular tonsillitis, may be accompanied by albuminuria in the absence of definite changes affecting the renal parenchyma. Its occurrence in an individual case is probably dependent to a very large degree upon the intensity of the intoxication. While it is generally an easy matter to distinguish between this form of albuminuria and that associated with distinct organic changes in the kidneys, considerable difficulty may at times be experienced; this question will be dealt with later on.

4. Albuminuria referable to Circulatory Disturbances.[3]—To this class belongs the albuminuria so frequently observed in cardiac insufficiency referable to valvular lesions, degeneration of the heart muscle from whatever cause, disease of the coronary arteries, etc., as well as in cases of impeded pulmonary circulation affecting the general circulation through the right heart, and, finally, in conditions associated with local circulatory disturbances, such as compression of the renal veins by a pregnant uterus, tumors, etc. It has been pointed out that febrile albuminuria also may, to a certain extent at least, be referable to such causes—*i. e.*, an ischemia or hyperemia of the kidneys produced by an increased or diminished blood pressure. The albuminuria observed in cases of cholera infantum, the simpler forms of intestinal catarrh, and in cholera Asiatica particularly, are undoubtedly dependent upon such causes. The quantity of albumin found under these circumstances varies considerably, but rarely exceeds 0.1 to 0.2 per cent. unless the disease has advanced to a stage where distinct changes in the renal parenchyma have resulted. The occurrence of albuminuria after cold baths, as stated above, is regarded by many as a "physiological" phenomenon, but this view should be rejected, as there can be little doubt that this form is also referable to circulatory disturbances.

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[1] Arnaud, "Albuminurie et lesions des reins dans la variole," Rev. d. méd., 1898, vol. xviii, p. 392.

[2] "Albuminuria in Plague," Brit. Med. Jour., Sept. 14, 1901.

[3] Senator, loc. cit.

5. Albuminuria referable to an Impeded Outflow of Urine.—Clinically, albuminuria referable primarily to an impeded outflow of urine from the kidneys is probably of more frequent occurrence than is generally supposed, and especially in women, in whom Kelly and others have demonstrated the frequent existence of ureteral stenoses. A complete blocking of the excretory duct, on the other hand, is rarely seen, but may be caused by the impaction of a renal calculus, the pressure of a tumor, or following certain gynecological operations in which the ureter is accidentally caught in a suture, etc. It has also been suggested that the albuminuria of pregnancy may be due to a compression of a ureter, but it is more likely that other factors are here at play.

6. Albuminuria of Hemic Origin.[1]—It was formerly supposed that Bright's disease was dependent upon certain abnormalities of the blood, and as a matter of fact this view has not only never been disproved, but is actually gaining ground from day to day. According to Semmola, Bright's disease is primarily due to an abnormal power of diffusion on the part of the albumins of the blood, which are eliminated by the kidneys as waste material. As a result of the excessive amount of work thus done, definite renal changes are finally produced. According to his theory, then, the albuminuria is the primary factor in the causation of nephritis. Should this hypothesis hold good, Senator is correct in asserting that an albuminuria of functional origin, so to speak, must precede the occurrence of the nephritis proper. He, however, doubts the occurrence of a prenephritic albuminuria; but others have noted the occurrence of definite renal changes which manifestly followed an apparently functional albuminuria (Da Costa). Further researches in this direction are urgently needed, and Semmola's view can at present only be regarded as an hypothesis. But even if such blood changes as those which Semmola suggests should not exist, there can be little doubt that true nephritis is dependent upon an acute or chronic dyscrasia of the blood, either in the sense of an abnormal mixture of the normal elements or of the presence of abnormal constituents. The same considerations undoubtedly also apply to various other forms of albuminuria, in so far as these are not the direct result of circulatory disturbances.

Clinically, albuminuria of hemic origin is observed in various diseases of the blood, such as purpura, scurvy, leukemia, pernicious anemia, as also in cases of poisoning with lead and mercury, in syphilis, jaundice, diabetes, following the inhalation of ether and chloroform, etc. The albuminuria associated with an excessive elimination of uric acid and oxalic acid, and, according to personal observations, with an excessive elimination of organic material in general, notably of urea, probably also belongs to this class.

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[1] v. Bamberger, loc. cit.

7. Toxic Albuminuria.—It has already been stated that the albuminuria of acute febrile diseases may, to a certain extent, be referable to a direct irritant action on the part of bacterial poisons upon the renal parenchyma. Poisoning with cantharides, mustard, oil of turpentine, potassium nitrate, carbolic acid, salicylic acid, tar, iodine, petroleum, phosphorus, arsenic, lead, antimony, alcohol, and mineral acids produces albuminuria. In all probability, however, the albuminuria here observed is referable not only to a direct irritant action upon the glandular epithelium of the kidneys, but also to circulatory disturbances.

8. Neurotic Albuminuria.—It is claimed by some that albumin, usually in small amounts, is eliminated in epilepsy after every attack, while others either deny its occurrence under such conditions or regard it as exceptional. In a number of cases in which I had occasion to examine urine voided after an attack, albumin was usually absent. It should be stated, however, that the seizures in these cases were comparatively slight, and that unfortunately an examination for semen was not made in those urines in which traces of albumin were demonstrated. An examination of the urine voided by a patient, after having been in the epileptic state for more than forty-eight hours, showed the presence of a small amount of albumin associated with an enormous elimination of uric acid, as well as a large excess of urea. Semen was absent.[1] Nothnagel states that he could not demonstrate any regularity in the appearance of albumin. In some of his cases with major attacks there was no albumin; in others it appeared after every attack; in still others it was sometimes present and at other times absent (in the same individual). At times it was found after a minor attack and was absent after a major attack (also in the same individual).

Other observers have obtained similar results, so that we may conclude that albuminuria following epileptic seizures is rather the exception than the rule. When it does occur, its significance is essentially the expression of a certain grade of cyanosis during the attacks.[2]

A transient albuminuria has also been noted in cases of progressive paralysis, mania, tetanus, delirium tremens, apoplexy, migraine, Basedow's disease, brain tumor, etc.

Although albuminuria may apparently be produced artificially by injuries affecting a certain area in the floor of the fourth ventricle analogous to the production of glucosuria (see Glucosuria), it would probably be going too far to assume the existence of a certain specific centre, stimulation of which causes the appearance of albumin in the urine. While the influence of the nervous system in preventing the passage of albumin through the glomeruli under normal conditions is undoubted, it would appear more likely that the albuminuria following injuries to the central nervous system is referable to circulatory disturbances in the kidneys secondary to lesions of the brain, and especially of the medulla. The albuminuria observed in certain neurotic individuals, on the other hand, is probably more frequently associated with metabolic abnormalities, and is of hemic origin.

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[1] M. Huppert, Virchow's Archiv, 1874, vol. lix, p. 305.

[2] Nothnagel, Ziemssen's Handbuch, 1877, vol. xii, p. 179. Binswanger, Nothnagel's spec. Pathol. u. Therap., vol. xii, p. 235 (literature).

9. A DIGESTIVE ALBUMINURIA has also been described.[1] It may follow the ingestion of excessive amounts of cheese, eggs—particularly when taken raw—beef, etc. Specially interesting is the form which follows the ingestion of excessive amounts of egg albumen. Ordinarily the consumption of a moderate amount of such albumen does not lead to albuminuria, while in cases of nephritis an already existing albuminuria is increased. But it has also been noted that even in individuals with *apparently* healthy kidneys, the ingestion of an excessive amount of egg albumen may call forth albuminuria, and it is possible in both cases to demonstrate the presence in the urine of both egg albumen and blood albumin.

To examine into this question the individual is given from four to eight raw eggs on an empty stomach in the morning for two to four days. His diet otherwise is as usual. The urine is collected at intervals of from two to three hours. If the ingestion of such an amount of egg albumen leads to albuminuria, this usually occurs after about four hours, and reaches its maximum intensity two hours later. Casts are not found (Jnouye).

The albuminuria in question, so far as the egg albuminuria goes, is undoubtedly owing to the fact that a certain amount of egg albumen is absorbed as such from the gastro-intestinal canal and is subsequently eliminated as foreign material. In what manner, however, the egg albuminuria may be responsible for the accompanying serum albuminuria is more difficult to explain.

Of the albuminuria which follows excessive indulgence in cheese and beef but little is known. Bearing in mind that the albuminuria very often follows the ingestion of such articles almost immediately, and before they have become absorbed, it is hardly justifiable to refer this form to the existence of a hyperalbuminosis. It would appear more rational, as Senator has suggested, to think of reflex vasomotor or trophic changes affecting the kidneys; while in other cases, in which the albuminuria does not follow the ingestion of such articles of food immediately, it is quite probable that it may be dependent upon certain metabolic abnormalities affecting the normal composition of the blood.

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[1] Ascoli, "Ueber d. Mechanismus d. Albuminurie durch Eiereiweiss," Münch. med. Woch., 1902, No. 10. Jnouye, "Ueber alimentäre Albuminurie," Deutsch. Arch. f. klin. Med., 1902, vol. lxxv, p. 378.

In the account thus given of the occurrence of albuminuria and its possible causes, reference has been had to only a *purely renal* albuminuria. It should be remembered, however, that the origin of the albumin may often be extremely difficult to determine, as albuminous material, such as blood and pus, may become mixed beyond the glandular portion of the kidneys with what would otherwise have been a perfectly normal urine, and that such an admixture may take place not only in the ureters, the bladder, and the urethra, but even in the pelvis of the kidney.

The term *accidental albuminuria* is applied to a condition in which albuminous material becomes mixed with a urine beyond the kidneys, as in cases of cystitis and urethritis, or whenever semen has entered the urine while the renal urine proper is free from albumin. An admixture of pus, blood, lymph, or chyle may, however, also occur in the kidneys, when the albuminuria is termed *accidental renal albuminuria*, an example of which is frequently seen in the slight degree of albuminuria referable to pyelitis during convalescence from typhoid fever. By a *mixed albuminuria* and a *mixed renal albuminuria*, on the other hand, we are to understand conditions in which the source of the albumin is twofold, renal and extrarenal in the first instance, parenchymal and extraparenchymal in the second, examples being the albuminuria of cystitis combined with nephritis and pyelonephritis, respectively.

It is manifest, of course, that in every instance in which albumin is found in the urine its origin should be ascertained. While this question is usually readily decided by a microscopic examination of the urine, considerable difficulty may occasionally be experienced. It is a well-known fact that in the urine of women a trace of albumin may frequently be detected, which is not due to any lesion of the urinary organs, but to an admixture of vaginal discharge, of blood during the process of menstruation, and, in married women, of semen. Whenever, therefore, doubt is felt as to the origin of the albumin, the specimen for examination should be obtained by the catheter, care being taken previously to cleanse the vulva. In men albumin may be referable to a gonorrheal urethritis. In such cases it is well to let the patient flush out his urethra first, and to make use for examination of the portion last voided. Very often, however, the conditions are more complex, it being uncertain whether the albumin is referable to the presence of pus only, or whether its origin is in the renal parenchyma. In such cases, as in cystitis, pyelonephritis, etc., a careful microscopic examination and enumeration of the pus corpuscles with the Thoma-Zeiss instrument are called for, and will in the majority of instances decide the question. Generally speaking, the amount of albumin found in uncomplicated cases of cystitis does not exceed 0.15 per cent., while in cases of pyelitis of the same intensity the amount of albumin is from two to three times as large.

Of late, attention has repeatedly been drawn to the occasional presence in the urine of an albuminous body which is soluble in acetic acid, and which Patein regards as a modification of common serum albumin. It has thus far been observed in only 8 cases, viz., twice in chronic nephritis, three times in eclampsia, once in a cystic kidney, once in tonsillitis following an injection of diphtheria antitoxin, and once in a pregnant woman in whom typhoid fever developed. I should suggest that the substance be spoken of as *Patein's albumin*[1] until its chemical identity has been established. The term *acetosoluble albumin* is, of course, likewise admissible.

So far as the *amount of albumin* is concerned, which may be eliminated in the twenty-four hours, an excretion of less than 2 grams may be regarded as insignificant, 6 to 8 grams as a moderate amount, and 10 to 12 grams or more as excessive. An excretion of 20 to 30 grams is exceptional.

Serum Globulin.—It has been pointed out that in cases of amyloid degeneration of the kidneys serum globulin is found in the urine together with serum albumin in large amounts, and, according to Senator, a ratio between the two albumins of 1 to 0.8 to 1.4 may be regarded as a fairly constant symptom of the disease, and of diagnostic importance. There seems to be no doubt, however, that serum globulin occurs in the urine, although in much smaller quantities than in the disease mentioned, whenever serum albumin is eliminated.[2]

A most remarkable instance of globulinuria has been recorded by Noel Paton,[3] in which the globulin separated out in crystalline form and was found in extraordinarily large quantity, amounting on one day to 70 grams.

Albumoses.—Albumoses have frequently been encountered in the urine, but are probably more frequently overlooked, as the bodies in question are not precipitated on boiling.

Albumosuria is observed under a great variety of conditions. It is thus noted in association with large accumulations of pus within the body, and there can be little doubt that the albumosuria is in such instances referable to a disintegration of the pus corpuscles and a resorption of the resulting albumoses. This form has hence been termed *pyogenic albumosuria.* It is principally observed during the stage of resolution in cases of croupous pneumonia; in association with pyothorax, and in cases of epidemic cerebrospinal meningitis, as contrasted with the tuberculous form. A *hepatogenic form* is noted in connection with diseases of the liver, notably acute yellow atrophy. Of its origin, however, nothing is known. Formerly, when the condition was looked upon as a peptonuria, and when it was thought that peptones were retransformed into native albumins in the liver, the "peptonuria" was explained upon the assumption that the liver had lost this power, and that the "peptones" accumulated in the blood, and were consequently eliminated in the urine. At the present day this view is no longer tenable.

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[1] Patein, "Acetosoluble Albumin in the Urine," Compt.-rend. de l'Acad. des sci., 1889. Coplin, Phila. Med. Jour., 1899, p. 957.

[2] Edlefsen, Deutsch. Arch. f. klin. Med., vol. vii, p. 67. Senator, Virchow's Archiv, vol. lx, p. 476. Petri, Diss., Berlin, 1876.

[3] B. Bramwell and N. Paton, Laboratory Reports of the Royal College of Physicians, Edinburgh, 1892, vol. iv, p. 47.

An *enterogenic form* of albumosuria has been noted in various diseases of the intestinal tract, such as typhoid fever, tuberculous ulceration, carcinoma, etc.; and it is possible that in these cases the albumoses are either directly absorbed from disintegrating pus, or that the intestine perhaps has in part lost the power of preventing the resorption of albumoses as such into the blood.

A *histogenic* or *hematogenic* origin has been ascribed to the albumosuria which is seen in cases of scurvy, in dermatitis, in various forms of poisoning, during the puerperal period and pregnancy, particularly following the death of the fetus, in various psychoses, in cases of carcinomatosis, acute yellow atrophy, etc.

A *renal* or *vesical form* of albumosuria is further noted in which the albumoses are derived from contained albumins, owing either to the presence of the common proteolytic ferments of the urine or to bacterial action, as in decomposing albuminous urines.

Aside from the conditions already mentioned, albumosuria has been observed in various septic conditions, in diphtheria, measles, scarlatina, acute articular rheumatism, mumps, malaria, phthisis; further, in association with leukemia, nephritis, puerperal parametritis, endocarditis, caries, pleurisy, heart disease, apoplexy, myxedema, carcinomatous peritonitis, in pneumonia, at the height of the disease and before resolution has set in, in liver abscess, etc.

In the differential diagnosis of suppurative meningitis a positive albumose reaction, according to Senator, speaks strongly in favor of the existence of this disease. In support of this view he cites the case of a young man, the subject of a median otitis of long standing, in which symptoms pointing to a meningitis—viz., fever, headache, and pains in the neck—were present, but in which no albumosuria was found to exist, and in which an operation revealed the presence of a cholesteatoma. A *digestive form* of albumosuria has recently been described, in which albumoses appear in the urine after their ingestion in large quantities, and it is claimed that this is observed only in cases of ulcerative disease of the intestinal tract. Only a positive result, however, is of value.

Very frequently albumosuria accompanies albuminuria, a condition which Senator has termed *mixed albuminuria*, and it is interesting to note that the albumosuria may alternate with the albuminuria, and may precede or follow the latter. In any case in which albumoses can be demonstrated in the urine the appearance of albumin should accordingly be anticipated.

In all cases of albumosuria the amount of albumose that appears in the urine is relatively small, and as a rule cannot be demonstrated by the biuret test when applied directly to the native urine. On the contrary, it is necessary to isolate the substance more or less definitely before deductions can be drawn as to its presence or absence.

LITERATURE.—Hofmeister, Prag. med. Woch., 1899, vol. v, pp. 321 and 325. v. Noorden, Lehrbuch d. Path. d. Stoffwechsels, Hirschwald, Berlin, 1893, p. 215. Senator, Deutsch. med. Woch., 1895, vol. xxi, p. 217. Stadelmann, Untersuchungen über Peptonurie, Bergmann, Wiesbaden, 1894. v. Jaksch. Prag. med. Woch., vol. v, pp. 292 and 303, and vol. vi, pp. 61, 74, 86, 133, 143; Zeit. f. klin. Med., 1883, vol. vi, p. 413. Krehl u. Matthes, Arch. f. klin. Med., 1895, vol. xlv, p. 54. Maixner, Zeit. f. klin. Med., 1884, vol. viii, p. 234. Fischel, Arch. f. Gynäk., 1884, vol. xxiv, p. 27. v. Jaksch, Prag. med. Woch., 1895, vol. xx, p. 430. Katz, Wien. med. Blätter, 1890, vol. xiv. L. v. Aldor, Berlin. klin. Woch., 1899, pp. 765 and 785.

Bence Jones' Albumin.—In association with the occurrence of multiple myeloma of the bones, notably when affecting the thoracic skeleton, a peculiar albuminous body may be found in the urine, which is apparently pathognomonic of the disease in question. It is to be noted, however, that cases have also been reported in which the substance was absent, so that a positive result only is of value. It was first observed by Bence Jones, and has heretofore been regarded as an albumose. From the researches of Magnus Levy and my own investigations, however, it appears that the substance is in reality a true albumin, as it yields a proto-albumose on peptic digestion; but it differs from all known albumins in its relative solubility on boiling, and in the readiness with which it dissolves in dilute ammonia after precipitation with alcohol. Like casein, it contains no hetero-group, but is distinguished from it by the presence of a carbohydrate radicle and the probable absence of phosphorus. It is crystallizable, and may occur in the urinary sediment in the form of typical spheroliths.

The amount of the substance which may be found in the urine is variable. Some observers have noted an elimination of from 0.25 to 6.0 pro mille, while others report much larger quantities. In Bence Jones' case the elimination rose on one occasion to 6.7 per cent., corresponding to a total output of 70 grams in the twenty-four hours—*i. e.*, to nearly as much as the entire amount of the albumins of the blood plasma.

As regards the origin of the albumin nothing definite is known, but there is reason to suppose that it is not derived from the myelomatous tissue as such. We may imagine, however, that through the agency of the cells of the abnormal tissue, viz., their products of metabolism, the normal transformation of the ingested albumins into tissue albumins is impeded, resulting in the production of the substance in question, which is then eliminated as foreign matter.

As the diagnosis of myeloma, in its early stages at least, is altogether dependent upon the demonstration of the albumin in question, a special examination should be made in this direction in all cases of obscure bone pain, as also in obscure cases of anemia, since Ellinger has shown that at times the disease may take its course without the occurrence of local symptoms, while a marked anemia may exist.

Of special interest in this connection is the fact that Zülzer claims to have succeeded in bringing about the appearance of Bence Jones' albumin in the urine of animals by feeding with pyrodin, which is known to be a distinct hemolytic poison.

It has been recorded by several observers that the Bence Jones albuminuria was accompanied by ordinary albuminuria. In no case, however, was the presence of common albumin established in a satisfactory manner, and it appears to me that its presence was merely assumed, whenever the urine did not clear entirely on boiling (see tests). This is unwarrantable, as it is now well known that the Bence Jones albumin itself, after being precipitated by heat, may not altogether dissolve on boiling. In two such cases, where one might have been led to assume the existence of ordinary albumin, I could demonstrate conclusively that this was not present. I should recommend that in all such cases the urine be carefully and slowly heated to 56° C., and maintained at that temperature until no more albumin separates out and that on cooling it be filtered. The filtrate can then be tested as usual for common albumin, either by heat or other tests, and I think that it will be found that common albumin is not present. That the two conditions *may* occur together is of course *a priori* possible, but in the previously recorded cases no satisfactory evidence has been brought forward to show that this did occur.

LITERATURE.—Bence Jones, Med. and Chir. Trans., 1850, vol. xxxiii; and Phil. Trans. Royal Soc. of London, 1848. Kühne, "Ueber Hemialbumose im Harn," Zeit. f. Biol., vol. xxix, p. 209. Ellinger, "Ueber d. Vorkommen d. Bence Jones'-schen Körper im Harn," Arch. f. klin. Med., 1898, vol. lxii, p. 255. Magnus Levy, Zeit. f. physiol. Chem., 1900, vol. xxx, p. 200. Hamburger, Johns Hopkins Hosp. Bull., Feb., 1901. Zülzer, Berlin. klin. Woch., 1900, p. 894. C. E. Simon, Amer. Jour. Med. Sci., 1902, vol. cxxiii, p. 954.

Peptonuria.—To judge from recent investigations by Ito,[1] true peptone in the sense of Kühne, may occur in the urine under pathological conditions. He obtained positive results in pneumonia, in advanced cases of phthisis, in ulcer of the stomach, and in several women after childbirth. The reaction was most intense in the pneumonia cases; it appeared already before resolution occurred, and disappeared a few days after the crisis. In the parturient women no reaction was obtained if the examination was delayed until after the tenth day. It is noteworthy that in the cases examined by Ito the peptonuria was always associated with the presence of albumoses (deutero-albumoses), and that the peptone was present in still smaller amount than the albumoses.

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[1] "Ueber d. Vorkommen v. echtem Pepton im Harn," Deutsch. Arch. f. klin. Med., 1901, vol. lxxi, p. 29.

Hemoglobin (Methemoglobin).—Under normal conditions the disintegration of the red blood corpuscles which is constantly taking place in the body never results in such a degree of hemoglobinemia as to be followed by an elimination of hemoglobin in the urine. Whenever the destruction of red corpuscles is so extensive, however, that the liver is unable to transform into bilirubin all the blood-coloring matter set free, *hemoglobinuria* occurs. While these factors, then—*i. e.*, an excessive destruction of the red blood corpuscles and an insufficiency on the part of the liver—must be regarded as explaining every case of hemoglobinuria, our knowledge of the ultimate causes of such excessive disintegration, as well as the manner in which these operate, is limited. Formerly the term *hematinuria* was applied to this condition. It was shown, however, that the pigment eliminated is in reality not hematin, but usually methemoglobin, and only at times hemoglobin, so that the term hemoglobinuria is also ill chosen.

Most common is the hemoglobinuria produced by certain poisons, such as potassium chlorate, arsenious hydride, hydrogen sulphide, pyrogallic acid, naphtol, hydrochloric acid, tincture of iodine, carbolic acid, carbon monoxide, etc., and also by morels (Helvella esculenta).

Quite familiar is the hemoglobinuria observed following transfusion of the blood of animals into man, such as that of the calf and lamb; also the form seen in extensive burns and in insolation.

While hemoglobinuria may occur in the course of any one of the specific infectious diseases, such as scarlatina, icterus gravis, variola hemorrhagica, typhoid fever, yellow fever, etc., it is said to be especially frequent in cases of malarial intoxication. This view is not accepted by many; Osler, among others, believes that it has frequently been confounded with malarial hematuria. I have never seen an instance of malarial hemoglobinuria, and believe that in our more temperate zones it scarcely ever occurs. Bastianello asserts that it is likewise rare in Italy, but more common in Sicily and Greece, and very common in the tropics. According to the same observer, hemoglobinuria occurs only in infections with the estivo-autumnal parasite. A hemoglobinuria due to quinine is likewise said to exist, but is certainly rare, excepting in patients who are suffering or have recently suffered from malarial fever. I have seen but one instance of hemoglobinuria following the ingestion of quinine. To judge from the literature upon the subject, there can be no doubt that syphilis may under certain conditions be a potent factor in the production of hemoglobinuria. This appears to be particularly true of those cases of so-called paroxysmal hemoglobinuria in which bloody urine is voided from time to time, the attacks being frequently preceded by chills and fever, so as closely to simulate malarial fever. Other factors, also, notably cold, appear to be concerned in the production of this form.

The occasional occurrence of hemoglobinuria in cases of Raynaud's disease, coincident with attacks of an epileptiform character, has been referred to in the chapter on the Blood.

Hemoglobinuria has been observed in a case of leukemia complicated by icterus.

Finally, an epidemic hemoglobinuria has been described as occurring in the newborn associated with jaundice, cyanosis, and nervous symptoms; of its causation we are in ignorance.

While hemoglobinuria is rather uncommon, *hematuria* is frequently observed, and will be considered later on, as its recognition is not dependent upon the demonstration of the albuminous body, "hemoglobin," alone in the urine, but upon the presence of red corpuscles, which in hemoglobinuria are either absent or present in only very small numbers.

LITERATURE.—Hemoglobinuria: Rosenbach, Berlin. klin. Woch., 1880, vol. xvii, pp. 132 and 151. Ehrlich, Zeit. f. klin. Med., 1881, vol. iii, p. 383. Boas, Arch. f. klin. Med., 1885, vol. xxxii, p. 355. Kobler u. Obermayer, Zeit. f. klin. Med., 1888, vol. xiii, p. 163.

Fibrin.—The occurrence of fibrin in the urine presupposes the presence of fibrinogen and a fibrinogenic ferment. It is seldom seen. According to Neubauer and Vogel, the fibrin may occur either as coagulated fibrin or in solution. In the former condition it is at times observed in the form of blood coagula, when its significance is essentially the same as that of hematuria in general, although it must be remembered that the usual form of hematuria is not associated with the presence of coagula. Colorless coagula of fibrin are seen in cases of chyluria or diphtheritic inflammation of the urinary passages. On the other hand, urines containing fibrinogenic material in solution are likewise seen but rarely, and are characterized by the fact that fibrinous coagula separate out only *on standing*, when they usually cover the bottom of the vessel; but at times they may change the entire bulk of urine into a gelatinous mass. This condition likewise is essentially observed in cases of chyluria, but may possibly also occur in association with nephritis. Lostorfer[1] has reported an instance of this kind, in which fibrinous coagulation took place in the *clear* urine, which contained much albumin, but no blood. Postmortem chronic inflammatory changes and amyloidosis of the kidney were found, while the urinary passages proper were intact.

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[1] Wien. klin. Woch., 1903, No. 7.

Nucleo-albumin.—The question whether or not nucleo-albumin is a normal constituent of the urine is still under dispute. Personal investigations have led me to the conclusin that with complicated methods and large amounts of urine—from 5 to 25 liters—it is always possible to demonstrate its presence both under physiological and pathological conditions. With the usual tests and smaller amounts of urine, however, negative results only are obtained in strictly normal individuals. According to my experience, trichloracetic acid, with which Stewart[1] claims to have obtained positive results in every one of the 150 normal urines which he examined, does not precipitate nucleo-albumin when this is present in normal amounts. *A nucleo-albuminuria recognizable by the available tests does not exist under normal conditions.* Even under pathological conditions nucleo-albumin is by no means always found. Sarzin[2] thus was unable to demonstrate its presence in 200 cases which he examined in Senator's clinic. Citron[3] arrived at similar results, and of several thousand urines which I have examined in this direction positive results were obtained in only a small percentage of cases. It is essentially met with in diseases which directly or indirectly involve the integrity of the epithelial lining of the uriniferous tubules or of the bladder. It has thus been frequently found in cases of acute nephritis and associated with febrile albuminuria, although its presence even then is not constant. In chronic nephritis it is more frequently absent than present. In cases of renal hyperemia and cystitis the results are variable. In 32 icteric urines Obermayer[4] obtained positive results without exception, and it appears that in leukemia nucleo-albumin is also quite constantly present. During the administration of pyrogallol, naphtol, corrosive sublimate, tar preparations, arsenic, etc., as well as in cases of poisoning with aniline and illuminating gas, large amounts of the substance may be found.

According to my experience, nucleo-albumin is frequently obtained in cases of so-called functional albuminuria, and it is not uncommon to find that this is still present when serum albumin and serum globulin can no longer be demonstrated, even with the trichloracetic acid test. Nucleo-albuminuria may thus exist independently of the presence of the more common forms of albumin. This observation has also been made by Strauss, who found nucleo-albumin only in several cases of cystitis, in one case of chronic interstitial nephritis, and in one case of emphysema pulmonum with renal hyperemia.

[1] Med. News, 1894.

[2] Ueber Nucleo-albuminausscheidung, Diss., Berlin, 1894.

[3] Ueber Mucin im Harn, Diss., Berlin, 1886.

[4] Centralbl. f. klin. Med., 1892, vol. xiii, p. 1.

The existence of a hematogenic form of nucleo-albuminuria has thus far not been satisfactorily demonstrated. It has been assumed that its presence indicates increased epithelial desquamation in some portion of the urinary tract—in other words, that it is of cellular origin. Matsumoto, however, has shown that even though a urine containing numerous epithelial casts, renal epithelial cells, and leukocytes be allowed to stand for some time, a substance which can be precipitated with acetic acid either does not occur at all or only in very small quantity. He has rendered it very probable that the substance which can be precipitated from pathological urines by means of acetic acid is largely fibrinogen and euglobulin. He adds that nucleo-albumin may be present simultaneously, but in comparison to the other two substances it is of secondary importance and is rarely seen.

Histon and Nucleohiston.—Kolisch and Burian[1] were able to demonstrate the presence of histon in a case of leukemia in which it was constantly present. More recently Krehl and Matthes[2] claim to have isolated the same substance in various febrile diseases, such as acute peritonitis, following appendicitis, in croupous pneumonia, erysipelas, and scarlatina. It is an albuminous body, and was first discovered by Kossel in the red blood corpuscles of the goose. It exists in the leukocytes of human blood in combination with the acid leukonuclein, constituting the so-called nucleohiston of Lilienfeld.

It is not clear in what manner the histonuria is produced; so much, however, seems certain, that it is not solely dependent upon increased destruction of leukocytes.

Nucleohiston itself has been found in the urine in a case of pseudoleukemia, by Jolles.[3]

Tests for Albumin.—The recognition of the various albuminous bodies which may occur in the urine is based partly upon their direct precipitation and partly upon color reactions when treated with certain reagents.

The number of tests which have from time to time been suggested is large; many of them after a brief period of use have been discarded as useless or uncertain, while others have been employed only occasionally, and have not received the recognition which they deserve, from the fact that simpler tests exist, that they do not possess sufficient delicacy, or that in some instances it is too great. In the following pages no attempt is made to describe all of these tests, and attention will be directed only to those which are generally used, and which clinical experience has proved to be of value, precedence being given to those which have been longest in use. While some of these are applicable for demonstrating the presence of more than one form of albumin, special tests will also be described whereby the various albumins may be individually recognized.

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[1] "Ueber d. Eiweisskörper d. leukämischen Harns," etc., Zeit. f. klin. Med., vol. xxix, p. 374.

[2] "Ueber febrile Albumosurie," Deutsch. Arch. f. klin. Med., vol. liv, p. 508.

[3] Ber d. deutsch. chem. Gesellsch., vol. xxx, p. 172; Zeit. f. klin. Med., vol. xxxiv, p. 53.

In every case the urine should be carefully filtered, so as to free it from any morphological elements, etc., present. To this end, it is generally sufficient to pass the urine through one or two layers of Swedish filter paper. Frequently, however, a clear specimen cannot be obtained in this manner; it is then advisable to shake the urine with burnt magnesia or talcum, or to mix it with scraps of filter paper, when it is filtered as usual.

Tests for Serum Albumin. THE NITRIC ACID TEST[1] (Plate XVIII). —The value of this test, properly applied, cannot be overestimated, as it is not only simple, but yields an amount of information that can otherwise be gained only with difficulty. Usually the student is advised to make use of a test-tube partially filled with urine, along the sides of which concentrated, chemically pure nitric acid is allowed to flow, so as to form a layer at the bottom of the tube, when in the presence of serum albumin a distinct white ring appears at the zone of contact between the two liquids (Heller's test). The pictures thus obtained cannot be compared, however, with those seen when the apparently trivial change is made of using a conical glass of about 2 ounces capacity instead of the test-tube. About 20 c.c. of urine are placed in the glass, when 6 to 10 c.c. of nitric acid are added by inclining the glass and allowing the nitric acid to flow down the sides. When this is carefully done the nitric acid forms a distinct zone beneath the urine. In the presence of albumin the white ring then appears, and varies in extent and intensity with the amount of albumin present. If now the contents of the glass are allowed to stand undisturbed—and if small amounts are present, the albumin appears on standing for a few minutes—it will be observed that the cloudiness gradually extends upward; and if much albumin is present, it may be seen to rise into the supernatant liquid in the form of small, irregular columns. This appearance is possibly referable to the decomposition of uric acid by means of nitric acid, nitrogen and carbon dioxide being set free, which, rising to the surface in the form of small bubbles, carry the nitric acid upward; coming into contact with albumin in solution, this is then precipitated.

In practically every urine on standing for a few minutes, a fine ring appears in the clear urine above or separated from the albuminous ring by a distinct clear layer of urine (Plate XVIII). This ring has been generally ascribed to the presence of urates and in certain hospitals of Paris it was long customary to gauge the amount of uric acid by the rapidity with which it forms and its extent. For years I regarded this as an established fact, but I have convinced myself

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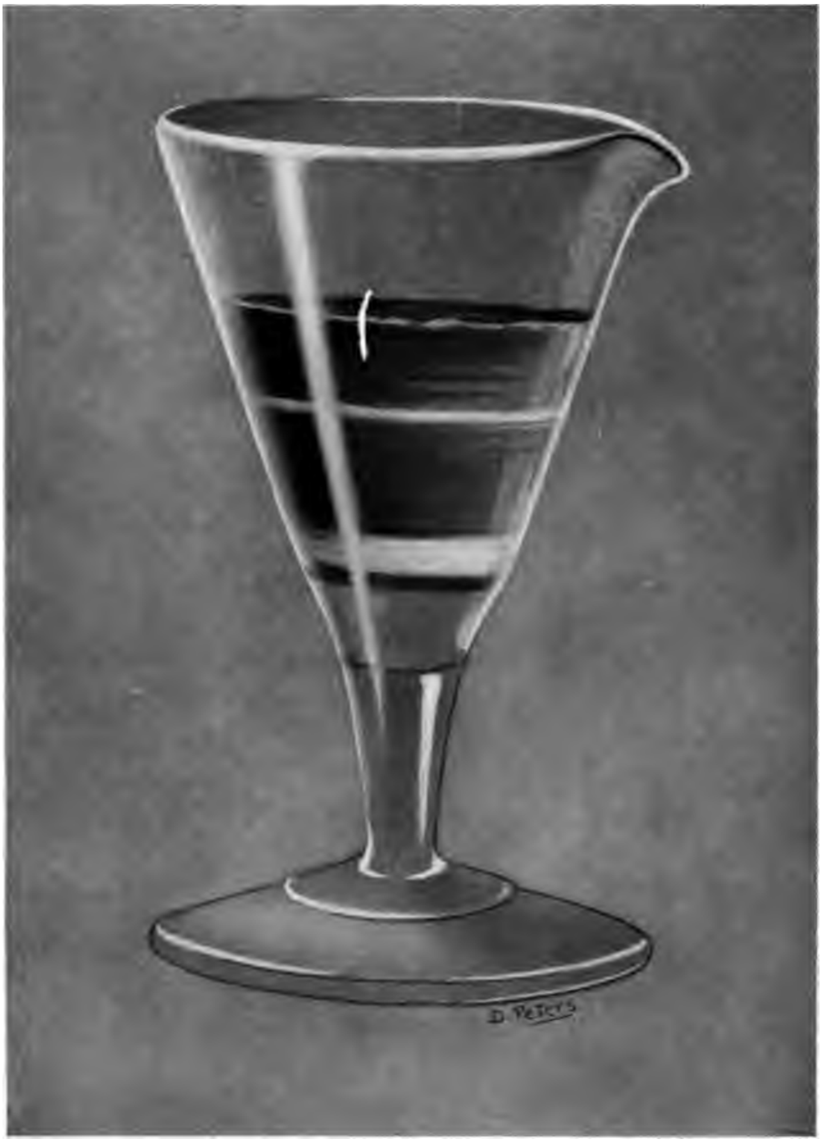
[1] J. F. Heller, Arch. f. physiol. u. path. Chem. u. Micros., 1852, vol. v, p. 169. A. Robin, Urologie clinique de la fièvre typhoide, Paris, 1877.

PLATE XVIII.



Cold Nitric Acid Test.

Albumin ring below; "urate" ring above.

that no relation exists between this phenomenon and the amount of uric acid, as determined by one of the standard methods. Mörner has expressed the opinion that the ring in question is not referable to urates at all, but is of a special albuminous character. Further researches in this direction are needed. Usually the ring is fine and delicate, but at times the substance is present in large amounts and may simulate common albumin, by rapidly extending downward. Its clinical significance is not understood.

Should more than 25 grams of urea be contained in a liter of the urine, an appearance like hoarfrost will be noted on the sides of the vessel, which is due to the formation of urea nitrate. Spangles of the same substance appear only in the presence of at least 45 grams; and if 50 grams or more of urea are contained in the liter, a dense mass of urea nitrate may be seen to separate out.

Biliary urine, when treated with nitric acid containing a little nitrous acid, shows the color play referable to the action of nitric acid upon bilirubin. The production of the colors (red, yellow, green, blue, and violet) takes place from above downward, the green color being the most characteristic; in the absence of the latter the presence of biliary pigment may be positively excluded. The presence of albumin does not interfere, as the color play takes place beneath the albuminous disk.

In normal urine a transparent ring is also obtained, presenting a peach-blossom red; the intensity of this may vary, however, from a faint rose to a pronounced brick color, and is referable to normal urinary pigment. In the presence of urobilin, on the other hand, this ring presents a distinct mahogany color.

Indican is indicated by the appearance of a violet ring situated above that referable to the normal urinary pigment. Its intensity varies with the amount present, from a light blue to a deep indigo.

The albumin ring at the zone of contact of the two fluids may be referable not only to the presence of serum albumin, but also of globulin and albumoses, while a negative reaction indicates the absence of these bodies. Should the precipitate caused by nitric acid consist of albumoses, it will clear up more or less, to reappear on cooling, the fluid at the same time assuming a markedly yellow color. The occurrence of a distinctly yellow color in the urine, moreover, which is only partially cleared upon the application of heat (and be it remembered that a much higher temperature is necessary for the solution of a precipitate referable to albumoses than of one due to urates), will indicate the existence of a mixed albuminuria—*i. e.*, the presence of coagulable albumin and albumoses.

Nitric acid may also cause a precipitation of certain resinous bodies, such as those contained in turpentine, balsam of copaiba and tolu, etc. If any doubt is felt, the mixture should be shaken with alcohol, when the precipitate caused by these substances is at once dissolved.

Nucleo-albumin, which is at times found in the urine, is also precipitated by nitric acid, but need not occupy our attention at this place. From what has been said, it is manifest that *the employment of the nitric acid test in the manner indicated furnishes much valuable information, and the adoption of the method as described not only by hospital students, but by general practitioners as well, cannot be too strongly urged.*

Boiling Test.—A few cubic centimeters of urine are boiled in a test-tube and then treated with a few drops of concentrated nitric acid, no matter whether a precipitate has occurred upon boiling or not. If albumin is present, this will separate out as a flaky precipitate, which consists of serum albumin frequently mixed with serum-globulin. It is true that albuminous urines will generally yield a precipitate on boiling alone; but it must be remembered that unless the reaction is decidedly acid a precipitation of normal calcium phosphate may occur, owing to the fact that the reaction of the urine upon boiling becomes less acid from the escape of carbonic acid held in solution. In urines presenting an alkaline or amphoteric reaction this is very frequently noted, and might give rise to confusion, as the precipitate due to calcium phosphate closely resembles that referable to albumin. In an alkaline medium, moreover, albumin may not be precipitated at all on boiling. Care must hence be taken to ensure a distinctly acid reaction, which is best accomplished by the addition of nitric acid, when a precipitate referable to phosphates is at once dissolved, while one due to albumin remains, and may even become more marked. The quantity to be added should usually be equivalent to about 0.05 to 0.1 per cent. of the volume of the urine. Under no condition should the acid be added before boiling, nor should the urine be boiled after its addition, as small amounts of albumin will otherwise be overlooked, owing to the fact that hot nitric acid dissolves the precipitate to a certain degree. If, after the addition of the nitric acid the urine turns a distinct yellow, and if then upon cooling a white precipitate appears, the presence of albumoses may be inferred. Uric acid will cause no confusion, as this separates out only upon cooling, and then presents a dark-brown color. As in the case of the nitric acid test, so also here, a precipitation of certain resins is noted at times which may be recognized by their solubility in alcohol. Albumoses are also precipitated upon the application of heat, but such precipitates again dissolve when the temperature approaches the boiling point.

Should acetic acid be used instead of nitric acid, great care must be taken to avoid an excess, as otherwise the albumin will be dissolved. As this danger diminishes the greater the quantity of salts contained in the urine, it is advisable to treat the urine first with a few drops of acetic acid until a distinctly acid reaction is obtained, and then to add one-sixth its volume of a saturated solution of sodium chloride, magnesium sulphate, or sodium sulphate, when upon boiling a precipitation of the albumin will occur. Carried out in this manner, the test is absolutely certain and will demonstrate even minimal amounts of albumin. If an equal volume of a saturated solution of common salt is added to the acidified urine, albumoses are also precipitated, but the precipitate dissolves on boiling.

THE POTASSIUM FERROCYANIDE TEST.—A few cubic centimeters of urine are *strongly* acidified with acetic acid (sp. gr. 1.064) and reated with a few drops of a 10 per cent. solution of potassium ferrocyanide, when, in the presence of but little albumin, a faint urbidity, or, if much albumin is present, a flaky precipitate, is noted, which is best recognized by comparison with a tube containing some of the pure filtered urine, both tubes being held against a black background. v. Jaksch advises the careful addition, by means of a pipette, of a few cubic centimeters of fairly concentrated acetic acid, to which a little potassium ferrocyanide has been added, when the albumin, as in Heller's test, is seen to form a ring at the zone of contact between the two fluids. Instead of potassium ferrocyanide, potassium platinocyanide may also be employed, and has the advantage that the test solution is colorless. Concentrated urines should be previously diluted with water. The presence of albumoses may be inferred if the precipitate disappears upon boiling, while a partial clearing up indicates the combined presence of albumoses and coagulable albumin.

At times the addition of acetic acid by itself is followed by the appearance of a cloud in the urine, which may be due to urates or o urinary mucin (nucleo-albumin), as already mentioned. In such cases the urine should be refiltered, diluted with water, and the test again applied; nucleo-albumin will dissolve in an excess of the acid.

THE TRICHLORACETIC ACID TEST.[1]—This test is undoubtedly he most delicate of those so far described, but not so delicate that a trace of albumin or nucleo-albumin can be demonstrated in every urine. An experience based upon the examination of several thousand urines with this reagent warrants my speaking with a certain degree of confidence upon the subject. Very frequently it is possible with this method to demonstrate albumin in urines in which he more common tests yield negative results, but in which tube casts may nevertheless be found upon microscopic examination. The test is applied as follows: By means of a pipette 1 or 2 c.c. of an aqueous solution of the reagent (sp. gr. 1.147) are carried to the bottom of a test-tube containing the carefully filtered urine, so as to orm a layer beneath the urine. In the presence of albumin a white ing will be seen to form at the zone of contact between the two fluids, varying in intensity with the amount of albumin present. So far as the test for albumin is concerned, this reagent possesses an advantage over nitric acid in that the colored rings, which are so confusing to the inexperienced, are commonly not observed. Serum albumin, serum globulin, and albumoses are precipitated, the presence of the latter being recognized, as in the previous tests, by the fact that the precipitate disappears upon boiling and reappears on cooling. A cloud, referable to uric acid (?), also appears if this is present in excessive amounts, but disappears upon the application of gentle heat. A previous dilution of the urine, moreover, guards against its occurrence.

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[1] F. Obermayer, Wien. med. Jahrbüch, 1888, p. 375. D. M. Reese, Johns Hopkins Hosp. Bull., 1890.

Other tests have also been suggested for the detection of albumin in the urine, such as the metaphosphoric acid test, the phenol, tannic acid, and picric acid tests, that with Tanret's reagent, phosphotungstic and phosphomolybdic acids, Spiegler's reagent, etc.

Of these, only the picric acid and Spiegler's test will be considered.

PICRIC ACID TEST.—The picric acid test is not applicable as a test for albumin as such, and is mentioned in this connection only because the same reagent is employed with Esbach's quantitative method. It is composed of 10 grams of picric acid and 20 grams of crystallized citric acid, dissolved in a liter of distilled water. If to this solution albuminous urine is added, the mixture is rendered turbid, and after some time a sediment which consists not only of albumins, but also of uric acid, kreatinin, and other extractives, will form at the bottom of the tube. (See Quantitative Estimation of Albumin.)

SPIEGLER'S TEST[1] (JOLLES' MODIFICATION).—The reagent consists of 10 grams of mercuric chloride, 20 grams of succinic acid, and 20 grams of sodium chloride, dissolved in 500 c.c. of distilled water. The urine is acidified to the extent of 5 c.c. with 1 c.c. of dilute acetic acid (30 per cent.), then superimposed by means of a pipette upon 4 to 5 c.c. of the reagent when in the presence of albumin a distinct white ring appears at the zone of contact. On warming, the precipitate does not disappear. As mucin is precipitated by the acid, it is well in doubtful cases to use for comparison 5 c.c. of the acidified urine, diluted with 4 to 5 c.c. of water. Albumoses and nucleo-albumin are also thrown down, and in the presence of iodides mercuric iodide is precipitated; the latter is soluble in alcohol.

SPECIAL TEST FOR SERUM ALBUMIN.—Should it be desired, for any reason, to demonstrate serum albumin alone, the urine is rendered amphoteric or faintly alkaline with sodium hydrate, and is then saturated with magnesium sulphate in substance, in order to remove any globulin. The filtrate is rendered distinctly acid with acetic acid, when a flaky precipitate, appearing upon boiling, will indicate the presence of serum albumin.

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[1] Wien. klin. Woch., 1892, vol. v, p. 26.

Patein's albumin differs from the common serum albumin in being soluble in acetic acid.[1]

Very often, as in the examination for sugar, it is necessary to remove any coagulable albumin that may be present, to which end the urine is rendered distinctly acid with acetic acid and boiled. An examination of the filtrate with potassium ferrocyanide, if the amount of acetic acid added was just sufficient, will then yield a negative result.

Quantitative Estimation of Albumin.—For the quantitative estimation of albumin a large number of methods have been devised, which fact in itself is sufficient to indicate that the majority of them, at least, are unsatisfactory.

Old Method by Boiling.—If comparative results only are desired, a definite amount of urine is boiled after acidifying with acetic acid; the albumin is allowed to settle for twenty-four hours. For this purpose Neubauer suggests the use of glass tubes measuring one-half to three-quarters of an inch in diameter, which are closed at the lower end with a cork. Ordinary test-tubes answer perfectly well, but care should be taken that the same quantity of urine is used in each case. The tubes are corked and kept for several days for comparison. The results, of course, express only the relative amount of albumin present, and it should be remembered that the error incurred may amount to as much as 30 or even 50 per cent. of the quantity that is found by gravimetric analysis. This is owing to the fact that sometimes the albumin separates out in large flakes, and at other times in small flakes, and that the degree of precipitation is also influenced by the specific gravity of the supernatant urine.

Esbach's Method.[2]—The reagent is composed of 10 grams of picric acid and 20 grams of citric acid, dissolved in 1000 c.c. of distilled water. Special tubes, termed albuminimeters (Fig. 147), are employed, which bear two marks, one, *U*, indicating the point to which urine must be added, and one, *R*, the point to which the reagent is added. The lower portion of the tube up to *U* bears a scale reading from 1 to 7, corresponding to the amount of albumin pro mille. The tube is filled to *U* with the filtered albuminous urine, and the reagent added until the point *R* is reached. The tube is closed with a stopper, inverted twelve times, and set aside for twenty-four hours.

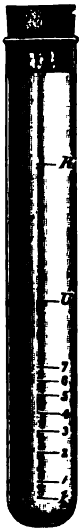

FIG. 147.—Esbach's albuminimeter.

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[1] Patein, "Acetosoluble Albumin in the Urine," Compt.-rend. de l'Acad. des sci., 1889. Coplin, Phila. Med. Jour., 1899, p. 957.

[2] Guttmann, Berlin. klin. Woch., 1886, vol. xxiii, p. 117.

At the expiration of this time serum albumin, serum globulin, and albumoses, as well as uric acid and kreatinin, will have settled, when the amount pro mille in grams may be read off from the scale. A few precautions must be observed in order to obtain as accurate results as possible. The reaction of the urine should be acid, and if this is not the case acetic acid is added. Its specific gravity should not exceed 1.006 or 1.008, the proper density being obtained by diluting with water. The amount of albumin in the specimen should not exceed 0.4 per cent.; if more be present, as determined by a preliminary test, the urine should be diluted. Most important, furthermore, is the temperature of the room. This should be 15° C.; variations from this point are apt to give rise to inaccurate results, which, according to Christensen, may amount to 100 per cent. in the case of a deviation of only 5° C. It is thus clear that as generally employed in the clinical laboratory the method will only give approximate results.

Gravimetric Method.—If accuracy is required the amount of albumin must be determined gravimetrically as follows: A certain quantity of urine, after having been acidified with an amount of acetic acid sufficient to ensure complete precipitation of all albumin, is boiled; the albumin is then filtered off, dried, and weighed. For this purpose, 500 to 1000 c.c. of filtered urine should be available. A specimen of this, if already acid, is placed in a test-tube, in boiling water, until coagulation takes place, when it is further heated over the free flame and filtered. The filtrate is tested with acetic acid and potassium ferrocyanide. Should no albumin be thus demonstrable, the entire amount of urine is treated in the same manner, and requires no further addition of acetic acid. If, however, the test yields a positive result, it is apparent that the urine was not sufficiently acid. The entire volume is then treated with a 30 to 50 per cent. solution of acetic acid, drop by drop, the mixture being thoroughly stirred and specimens tested from time to time, as described. When, finally, the urine remains clear or shows only a faint turbidity, 100 c.c. or less, according to the amount of albumin present, are first heated in boiling water until the albumin begins to separate out in flakes, and then brought to the boiling point over the free flame. The supernatant urine is decanted through a filter, which has been previously dried at 120° to 130° C. and accurately weighed, when the whole amount of the precipitate is brought upon the filter. Any albumin remaining in the beaker is detached from its sides by means of a glass rod tipped with a piece of rubber tubing, and collected by the aid of hot water. The entire precipitate is thoroughly washed with hot water until the washings no longer become turbid when treated with a drop of nitric acid and silver nitrate; in other words, until the chlorides have been removed. The precipitate is further washed with alcohol and finally with ether to remove any fats that may be present, when it is dried at 120° to 130° C. to a constant weight. If still greater accuracy is required, the dried and weighed precipitate is incinerated to determine the amount of mineral ash in combination with the albumin, which is then deducted from the total weight. The most accurate results are obtained if not more than 0.2 to 0.3 gram of albumin is contained in the amount of urine employed. A smaller quantity than 100 c.c. should hence be used if a previous test with Esbach's albuminimeter shows a higher percentage.

A glass-wool filter ensures a more rapid process of drying—twenty-four to thirty hours; but care must be had that this is properly prepared, so as to guard against a loss of the wool while washing.

Method of Centrifugation.—This presupposes a constant speed, and hence an electrical centrifuge is a prerequisite, which is an objection to the general adoption of the method. Approximative results only are obtained.

Test for Serum Globulin and its Quantitative Estimation.—To test for serum-globulin the urine is rendered alkaline by the addition of ammonium hydrate, any phosphates that may thus be thrown down being filtered off on standing. The urine is then treated with an equal volume of a saturated solution of ammonium sulphate, when the occurrence of a precipitate will indicate the presence of globulins. Ammonium urate may likewise separate out, but this occurs later.

According to Paton, the following test may also be employed: The urine after having been rendered alkaline with sodium hydrate—any phosphates which may separate out are filtered off—is carefully poured down the side of a test-tube containing a saturated solution of sodium sulphate, so as to form a layer above this, when in the presence of serum globulin a white ring will appear at the zone of contact.

If a *quantitative estimation* of the globulin is to be made, the precipitate thus obtained, after about one hour's standing, is collected on a dried and weighed filter, and washed thoroughly with a one-half saturated solution of ammonium sulphate until a specimen of the washings treated with acetic acid and potassium ferrocyanide no longer gives a precipitate. It is then treated as directed in the method employed for the quantitative estimation of serum albumin.

Tests for Albumoses.—A small amount of urine is strongly acidified with acetic acid and treated with an equal volume of a saturated solution of common salt. In the presence of albumoses a precipitate occurs, which dissolves on boiling and reappears on cooling. If serum albumin also be present, which is usually the case, the hot liquid must be filtered. The albumoses are found in the filtrate and appear on cooling. If the *hot* filtrate, moreover, is rendered strongly alkaline with a solution of sodium hydrate, a red color develops upon the addition of a very dilute solution of cupric sulphate (1 to 2 per cent.), added drop by drop (biuret reaction). On boiling with *Millon's reagent* a red color is also obtained. This reagent is prepared by dissolving 1 part of mercury in 2 parts of nitric acid of a specific gravity of 1.42, and diluting with 2 volumes of distilled water.

Bang's Method.—10 c.c. of urine are heated in a test-tube with 8 grams of finely powdered ammonium sulphate until the salt has been dissolved; the fluid is then boiled for a moment. The hot fluid is centrifugated for one-half to one minute, the supernatant fluid poured off, and the sediment stirred with alcohol in an agate mortar. The alcohol is poured off, and the residue dissolved in a little water; the solution is boiled and filtered, and the filtrate tested with sodium hydrate solution and cupric sulphate as described above. Should the urine be rich in urobilin—*i. e.*, manifesting a well-marked fluorescence with zinc chloride and ammonia—it is best to extract the final aqueous solution with chloroform by shaking, and to pour off the supernatant fluid, when this is tested with cupric sulphate. In this manner it is possible to demonstrate the presence of albumoses in a dilution of 1 in 4000 to 5000. Other constituents of the urine, with the exception of hematoporphyrin, do not interfere with the test. Should hematoporphyrin be present, however, which may be suspected if a red alcoholic extract is obtained, the urine must first be precipitated with barium chloride. The filtrate, which contains the albumoses, is then examined as described.

If a centrifuge is not available, the urine is boiled with the ammonium sulphate, when a portion of the albumoses will remain on the sides of the tube as a sticky mass. This is washed with alcohol, and if necessary with chloroform, dissolved in water, and tested for biuret.

The alcoholic extract may also be used for testing for urobilin. To this end it is only necessary to add a few drops of a solution of zinc chloride, when in the presence of urobilin a beautiful fluorescence will be observed. The test is extremely delicate.[1]

Examination for True Peptone[2] (**Polypeptids**).—To demonstrate the presence of true peptone (in the sense of Kühne) in the urine, about 300 c.c. of filtered acid urine are saturated on a water bath with ammonium sulphate at a temperature between 60° and 70° C. On cooling, the mixture is filtered, the filtrate is alkalinized with a dilute solution of sodium carbonate, again saturated between 60° and 70° C. with ammonium sulphate, filtered on cooling, the filtrate neutralized with very dilute acetic acid, again saturated with the salt between 40° and 50° C., and finally again filtered on cooling. The final filtrate is diluted with an equal volume of distilled water and ıted with a freshly prepared solution of tannic acid, which is led drop by drop, care being taken to avoid an excess. The cipitate is filtered off the next day, dried in the desiccator upon filter, powdered, and covered in a porcelain crucible with a ıll amount of baryta-water to which a little finely powdered baryta ıdded. The mixture is placed on a boiling water bath for three ıutes, and after one or two hours it is filtered. If necessary, the ıtion is decolorized with neutral lead acetate. The biuret test inally applied, and if positive indicates the presence of peptone the sense of Kühne.

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[1] E. Bang, "Eine neue Methode zum Nachweis d. Albumosen im Harn," Deutsch. med. Woch., 1898, p. 17.

[2] Ito, loc. cit

'ests for Bence Jones' Albumin.—The presence of Bence Jones' umin is usually discovered on slowly heating the urine to the ling point. It will then be noted that at a temperature of from to 60° C. a more or less intense, milky turbidity develops, which subsequent boiling either disappears entirely or partially, and ppears on cooling. The degree to which the urine clears on ling differs in different cases. As I have just stated, the turbid- may disappear entirely; but, on the other hand, urines are met h in which even a partial clearing can scarcely be made out. is is apparently dependent upon the degree of acidity of the urine, amount of mineral salts and of urea present, and probably also ›n other and still unknown factors; it does not necessarily indicate t common albumin is simultaneously present.

Jpon the addition of a drop of nitric acid to a few cubic centi- ters of such urine a temporary turbidity develops, which disap- .rs on shaking, but persists if a little more of the acid is added. ıow the mixture is heated, the albumin first coagulates to a dense ss; on boiling, this dissolves, and after a while the liquid becomes ıost entirely clear, while the turbidity returns, as before, on sub- uent cooling. Similar reactions are obtained with all the common gents for albumin.

'or its complete identification, the albumin should be isolated l further examined as follows: Large amounts of urine are pre- itated by the addition of one and one-half to two volumes of per cent. alcohol, or by treating with two volumes of a saturated ıtion of ammonium sulphate. In either event the total amount ılbumin is thrown down. This is then washed with alcohol and er, and dried over sulphuric acid. To purify the substance it is solved in boiling water, by the aid of a few drops of a dilute ıtion of sodium carbonate, and dialyzed to running and then to tilled water until free from mineral salts. It is then reprecipi- ed with alcohol (if necessary, after the addition of a drop or two a dilute solution of hydrochloric acid), washed with absolute ohol and ether, and dried. Thus purified, the albumin is prac- ılly insoluble in distilled water or saline solution at ordinary tem- ature, and only sparingly so at the boiling point. In boiling water, however, it dissolves with comparative ease after the addition of a few drops of sodium carbonate solution. On neutralization no precipitate occurs if a sufficient amount of water is present. If such a neutral solution is heated, no change occurs; but if it is now acidified and a certain amount of salt added, the typical reaction appears on heating, viz., precipitation between 50° and 60° C. (even between 40° and 50° C. if a sufficient amount of salt is present), clearing on boiling, and reprecipitation on cooling.

On digestion with pepsin-hydrochloric acid a proto-albumose is obtained among the early products of digestion, while a hetero-albumose is not formed. (See Bence Jones' Albumin, p. 456.)

Boston's suggestion that the albumin in question can be recognized from its higher content of loosely combined sulphur, by qualitative examination, does not seem to the writer to be of value.

Test for Nucleo-albumin.—It has been generally supposed that the substance which is precipitated on adding strong acetic acid to certain pathological urines, when diluted two or three times with water, is nucleo-albumin, the precipitate being soluble or largely so in an excess of the reagent. Matsumoto,[1] however, has recently pointed out that the substance which is precipitated in this manner is largely a mixture of fibrinogen (fibrinoglobulin) and euglobulin. Nucleo-albumin may be present at the same time, but it is rare, and its quantity in comparison to the two albumins mentioned insignificant.

To demonstrate the presence of nucleo-albumin, it is necessary to salt out the albumins with ammonium sulphate (half saturation is sufficient), and then to ascertain whether any precipitation occurs within the limits of precipitation of nucleo-albumin. Matsumoto gives these as 0.1 to 0.8 (lower limit) and 1.6 and 2.2 (upper limit). Its limits of precipitation are the lowest of the known albumins.[2]

Whether or not *Ott's test*[3] in the light of this work can still be relied upon as a test for the demonstration of nucleo-albumin may be questioned. It is conducted as follows: A few cubic centimeters of urine are treated with an equal volume of a saturated solution of common salt, when Almén's solution, which consists of 5 grams of tannic acid, 10 c.c. of a 25 per cent. solution of acetic acid, and 240 c.c. of 40 to 50 per cent. alcohol, is slowly added. The development of a precipitate was regarded as evidence of the presence of nucleo-albumin.

In order to remove nucleo-albumin from the urine, this is treated with neutral lead acetate, an excess of the reagent being avoided.

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[1] Ueber d. durch Essigsäure ausfällbare Eiweissubstanz in pathologischen Harnen, Deutsch. Arch., 1902, vol. xxv, p. 398.

[2] Limit of precipitation of fibrinogen, 1.5 to 1.7 to 2.5 to 2.7; of fibrinoglobulin, 2.2 to 2.9; of euglobulin, 2.8 to 3.3; of pseudoglobulin, 3.4 to 4.6.

[3] Centralbl. f. inn. Med., 1895, vol. xvi, p. 38.

Test for Hemoglobin.—The diagnosis of hemoglobinuria is based upon the demonstration of hemoglobin, viz., methemoglobin, in the urine in solution, in the absence of red corpuscles, or at least in the presence of only a very small number.

Bloody urine is generally turbid, and may vary in color from bright red to almost black.

Oxyhemoglobin, as such, can only be recognized by the spectroscope; it gives rise to the appearance of two bands of absorption, situated between D and E, as described in the chapter on the Blood.

The urine to be examined spectroscopically should be rendered feebly acid by means of acetic acid, and placed before the open slit of the spectroscope in a test-tube, beaker, or similar vessel, when the two bands of oxyhemoglobin will be seen, either at once or upon diluting with distilled water. If ammonium sulphide is added, the spectrum of reduced hemoglobin will be obtained. It must be remembered that more commonly the spectrum of methemoglobin is seen in cases of hemoglobinuria.

The following tests, which will also indicate the presence of blood-coloring matter, cannot be employed to decide the nature of the pigment present, as methemoglobin and oxyhemoglobin will both react in the same manner.

Heller's Test.[1]—A small amount of the urine, or still better a portion of the sediment, is made strongly alkaline with sodium hydrate and boiled. On standing, a deposit of basic phosphates forms, which in the presence of blood-coloring matter presents a bright-red color. This is referable to the formation of hemochromogen, as may be shown by spectroscopic examination. Thus controlled, the test is extremely sensitive, and still yields a positive result when the chemical test alone leaves one in doubt.[2] The deciding band is the first between D and E. Care should be had, however, that the solution is cold, as otherwise the hemochromogen is transformed into hematin in alkaline solution. At times, when the urine contains a large amount of coloring matter (bile pigment, etc.), it may be difficult to determine the exact color of the sediment. In such cases the subsequent examination with the spectroscope—the lensless instrument of Hering or that of Browning suffices—is invaluable. In the absence of such apparatus the procedure of v. Jaksch may be employed. To this end the phosphatic deposit is filtered off and dissolved in acetic acid, when if blood pigment is present the solution becomes red, the color gradually vanishing upon exposure to the air. The delicacy of the test is such that oxyhemoglobin can still be demonstrated in a dilution of 1 to 4000.

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[1] Zeit. d. K. K. Gesellsch. d. Aerzte zu Wien, 1858, No. 48.

V. Arnold, Berlin. klin. Woch., 1898, p. 283.

Donogany's Test.[1]—About 10 c.c. of urine are treated with 1 c.c. of a solution of ammonium sulphide and the same amount of pyridin, when in the presence of blood a more or less intense orange color develops, especially if looked at from above, against a white background. In doubtful cases the examination is to be controlled by a spectroscopic examination of the resulting mixture. If blood pigment is present, the spectrum of hemochromogen is obtained. Should the ammonium sulphide and pyridin be old, a green or brown color is imparted to the urine, which changes to yellow upon the addition of ammonium hydrate.

Test for Fibrin.—Fibrin usually occurs in the urine in the form of distinct clots, the nature of which may be determined by thoroughly washing with water, when they are dissolved by boiling in a 1 per cent. solution of soda or a 5 per cent. solution of hydrochloric acid. On cooling, this solution is tested as for serum albumin.

Test for Histon.—The urine of twenty-four hours is first examined for albumin, and this removed if present. It is then precipitated with 94 per cent. alcohol, the precipitate washed with hot alcohol and dissolved in boiling water. Upon cooling, the solution thus obtained is acidified with hydrochloric acid and allowed to stand for several hours. During this time a cloudiness, referable to a large extent to uric acid, develops, which is filtered off, and the filtrate is precipitated with ammonia. The precipitate is collected on a small filter and washed with ammoniacal water until the washings no longer give the biuret reaction. It is then dissolved in dilute acetic acid and the solution tested with the biuret test; if this yields a positive result, and if coagulation occurs upon the application of heat, the coagulum being soluble in mineral acids, the presence of histon may be inferred.

Carbohydrates.

Glucose.—Through the researches of Wedenski, v. Udranszky, and others,[2] we know that traces of glucose may be encountered in the urine under strictly normal conditions. The amount, however, is extremely small, and special methods are necessary in order to demonstrate its presence. With the usual clinical tests normal urine is apparently free from sugar unless unduly large amounts have recently been ingested. In that event a certain amount of glucose is eliminated in the urine, constituting the so-called *digestive glucosuria* of Claude Bernard.[3]

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[1] "Darstellung d. Hæmochromogen als Reaction auf Blut," etc., Virchow's Archiv, vol. cxlviii, p. 234.

[2] A. Baumann, Br. d. Deutsch. chem. Ges., 1886, vol. xix, p. 3218. N. Wedenski, Zeit. f. physiol. Chem., 1889, vol. xiii, p. 122. K. Baisch, ibid., 1894, vol. xviii, p. 193, and 1895, vol. xix, p. 348.

[3] Compt.-rend. de l'Acad. des sci., 1859, vol. xlviii, p. 673

The normal limit to the assimilation of glucose on the part of the body economy is subject to considerable variation. Some observers thus report that the ingestion of such large amounts as 200 and 250 grams does not lead to glucosuria, while others have found sugar in the urine after the administration of 100 grams. In view of the possible relation existing between diabetes and a lowered limit to the assimilation of glucose in apparently normal individuals, or at least in persons in whose urine glucose cannot be constantly demonstrated, this question has created much interest within the last few years and has called forth a large amount of work. The majority of investigators are now in accord in regarding as abnormal a glucosuria that follows the ingestion of 100 grams of chemically pure glucose.

The method usually employed in order to ascertain the power of assimilation for glucose on the part of an individual is the following:

The patient receives 100 grams of glucose, in substance, dissolved in 500 c.c. of water, on an empty stomach, and is instructed to pass his water hourly during the following four to five hours. During this time, moreover, no food is to be taken. The individual specimens, as well as the urine which has been passed during the night, are then tested with Trommer's and Nylander's tests, with the fermentation test, and with phenylhydrazin. A positive result, however, is recorded only when sugar can be demonstrated with the fermentation test.

Cane sugar and larger amounts of glucose have also been used; but it is better, on the whole, as Strauss has pointed out, to give glucose, and not to exceed the dose of 100 grams.

Especially interesting are the results which have been obtained in various diseases of the liver, to which organ the important function of preventing an undue accumulation of sugar in the blood has been repeatedly ascribed. Bierens de Haën[1] thus reports that of 29 cases of various hepatic diseases he found sugar in 18 after the administration of 150 grams of cane sugar; and v. Jaksch[2] claims to have obtained positive results in 15 cases of phosphorus poisoning out of 43. Strauss,[3] on the other hand, states that he found sugar in only 2 of his 38 cases, and has collected 107 additional cases from the literature, in only 14 of which could sugar be demonstrated. If we add these together, we have 145 cases of various hepatic diseases, with negative results in 88.9 per cent. Referring to the contradictory results obtained, Strauss points out that these may have been accidental in part, but that the interpretation which has been offered by v. Jaksch and de Haën may not have been correct. It is thus possible that in his cases of phosphorus poisoning other factors besides the changes in the liver, such as the action of the poison upon the nervous system, etc., played a *role*, as a digestive glucosuria may also occur in connection with other forms of intoxication, as in fevers, following the administration of large doses of diuretin, in acute alcoholism, etc., in which the liver is not the only organ that is involved. Strauss further shows that great care must be exercised in the selection of the material for such investigations, and believes that errors referable to this source may have been incurred by Bierens de Haën. He thus cites 2 cases of hypertrophic cirrhosis, associated with delirium tremens, in which small amounts of sugar could be demonstrated in the urine a few days after recovery from the delirium, while shortly after negative results only could be obtained. The lowering effect of alcoholism upon the limit to the assimilation of glucose is a well-known phenomenon, and it would be erroneous to conclude that because alcoholism may call forth organic changes in the liver the digestive glucosuria in such cases must be referable to such alterations. Without entering further into the question at this place, it appears that diseases of the liver *per se* do not materially lessen the power of assimilation for glucose, and that other forces are at the disposal of the body to supply the glycogen-forming or retaining power of the liver when this becomes insufficient, and that these also may be at fault when a digestive glucosuria is observed in association with hepatic disorders.

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[1] "Ueber alimentäre Glycosurie bei Leberkranken," Arch. f. Verdauungskrank., vol. iv, p. 4.

[2] "Alimentäre Glycosurie," Prag. med. Woch., 1895, Nos. 27, 31, and 32.

[3] "Leber und Glycosurie," Berlin. klin. Woch., 1898, p. 1122.

The association of digestive glucosuria with various diseases of the nervous system has been carefully studied by v. Jaksch,[1] Strümpell, H. Strauss,[2] von Oordt, Geelvink, and Arndt.[3] From the work of these investigators it appears that digestive glucosuria is rarely seen in spinal diseases, and is decidedly more common in functional diseases of the central nervous system than in organic affections. Of 30 cases of tabes examined by Strauss, digestive glucosuria resulted in only 1 after the administration of 100 grams of glucose, and in that case a family history of diabetes existed. In 16 further cases examined by J. Strauss negative results were obtained. In the neuroses a positive result was noted in 42 out of 210 cases which I have been able to collect from the literature. Most frequently it is met with in the traumatic neuroses, in whch Strauss observed the phenomenon in 37.5 per cent. of his 40 cases; while in the non-traumatic forms only 14.4 per cent. were insufficient in this respect. Of the organic diseases of the central nervous system, it appears that diffuse cerebral lesions referable to alcohol and syphilis are more likely to give rise to this form of glucosuria than the more localized lesions. In general paresis digestive glucosuria is thus not uncommon (H. Strauss, Arndt), but it is only possible to draw definite deductions from the study of a large amount of clinical material. Small series like that of J. Strauss do not give a proper idea of actual conditions, as he, for example, obtained negative results in all of 10 cases.

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[1] Loc. cit.

[2] "Zur Lehre v. d. neurogenen u. d. thyreogenen Glycosurie," Deutsch. med. Woch., 1897, pp. 275 and 309.

[3] "Ueber alimentäre Glycosurie bei Neuropsychosen," Berlin. klin. Woch., 1898, p. 1085.

In his examination of 5 cases of idiocy and 23 cases of imbecility, J. Strauss obtained positive results in only 2 of the imbeciles after the administration of 100 grams of glucose; in both of the positive cases the glucosuria was transitory and associated with the existence of nervous excitability. Bergenthal observed alimentary glucosuria in 6 cases out of 20.

In Basedow's disease digestive glucosuria has also been noted in a large number of cases by Chvostek, Kraus and Ludwig, Strauss, Goldschmidt and Stern. Especially interesting in this connection is the fact that digestive glucosuria may be induced by the administration of thyroid extract, viz., thyroidin or iodothyrin in apparently normal persons. Bettmann[1] thus noted glucosuria after the ingestion of 100 grams of glucose in 12 of 25 healthy individuals who had been treated for a week with the products in question.

A digestive glucosuria is further observed in numerous febrile diseases, such as pneumonia, typhoid fever, acute articular rheumatism, scarlatina, tonsillitis, etc. The amount of sugar usually found varies from 0.5 to 3 per cent.; larger amounts may, however, also be encountered, and 1 case is on record in which 8 per cent. was present.[2]

Very common also, as I have indicated, is the digestive glucosuria of alcoholics, and there can be little doubt that the habitual ingestion of large quantities of beer and spirits is apt in the course of time to lead to a more than temporary insufficiency of the carbohydrate metabolism. In the course of his investigations in this direction, Krehl[3] found among the Jena students that the proportion of those in whose urine sugar appeared apparently varied with the different kinds of beer, but was much greater after morning drinking. Of 14 who drank bock or export beer in the morning, 5 had glucosuria. After the evening drinking, amounting in 1 case to seven liters, of 19 only 1 had sugar in the urine, and with Bavarian beer 1 of 11.

Of diseases of the skin, digestive glucosuria is notably associated with psoriasis; and it is interesting to note that the same disease is not infrequently seen in diabetic patients. Gross thus records 5 cases, in 4 of which the psoriasis had existed for many years before the appearance of diabetic symptoms. Similar instances are recorded by Strauss, Grube, Polotebuoff, Nielssen, Schütz, and others. Nagelschmidt[1] was able to produce glucosuria by the ingestion of 100 grams of glucose in 8 cases out of 25.

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[1] "Ueber d. Einfluss d. Schilddrüsenbehandl. auf. d. Kohlendydratstoffwechsel,' Berlin. klin. Woch., 1897, p. 518.

[2] R. v. Bleiweiss, "Ueber alimentäre Glycosurie e saccharo bei acuten, fieber aften Infektionskrankheiten," Centralbl. f. inn. Med., 1900, No. 2.

[3] "Alimentäre Glycosurie nach Biergenuss," Centralbl. f. inn. Med., 1897, No. 40

During pregnancy digestive glucosuria is also frequently observed, and is by some regarded as a fairly constant symptom and of diagnostic importance. The amount is variable, and while Lanz[2] records 1 case in which 29.6 grams of glucose were found after the ingestion of 100 grams, such figures are certainly uncommon, and as a general rule less than 3 grams are recovered from the urine. After delivery the power of assimilation for glucose no longer appears to be subnormal. The milder form of glucosuria in pregnancy is during the last week or two accompanied by lactosuria.

A digestive glucosuria has further been observed in acute and chronic lead poisoning, poisoning with nitrobenzol, aniline dyes, opium, atropine, and carbon monoxide; in the early stages (the first twelve days) of acute phosphorus poisoning; in the febrile form of *embarras gastrique*, etc. In these conditions, however, the phenomenon has received little attention.

In patients afflicted with disease of the heart, liver, and kidneys Gobbi[3] observed a digestive glucosuria, after the ingestion of from 100 to 200 grams of glucose, if diuretin was at the same time administered.

Very important is the fact that in diabetes mellitus the sugar may at times disappear from the urine, while its elimination is replaced by an excessive excretion of uric acid or phosphates. In such cases glucosuria may be produced with ease by the ingestion of 100 grams of glucose, a point which may be of value in diagnosis. The exhibition of such amounts of sugar in true diabetes while glucosuria already exists will cause an increased elimination, while this apparently does not occur in other forms of glucosuria. Interesting further is the fact that in diabetic patients an increased elimination of sugar can be produced by the administration of full doses of copaiba. That this drug is in itself capable of lowering the limit of the assimilation of glucose has recently been shown by Bettmann. A digestive glucosuria was thus produced in 4 patients out of 12 of whom copaiba had been given for one week in amounts varying from 1 to 2 grams.

The digestive glucosuria to which reference has been made in the preceding pages is generally spoken of as the *digestive glucosuria e saccharo*. Similar results have been obtained after the administration of starches in excess, viz., 150 to 200 grams. But while a digestive glucosuria e saccharo is regarded only as a possible indication of a pathological alteration of the carbohydrate metabolism, it is generally thought that every *glucosuria ex amylo*[1] is indicative of a definite disturbance in the sense of diabetes, unless special factors, such as an increase of the surrounding temperature, diminished radiation of heat, or complete lack of muscular activity, are active. Strauss, however, hȧs shown that in cases in which a somewhat more than temporary predisposition toward glucosuria e saccharo exists, as in alcoholics, for examplee, a coincident tendency toward glucosuria ex amylo may likewise be demonstrated. As a result of his experiments he concludes that the difference between the digestive glucosuria e saccharo and glucosuria ex amylo is essentially a question of degree. *Cæteris paribus,* it appears that harmful influences of a slight character lead to glucosuria e saccharo, while grave insults call forth glucosuria ex amylo. It results practically that the prognosis in those cases in which digestive glucosuria follows a temporary insult is far better than when the carbohydrate metabolism is permanently damaged, and especially when a glucosuria ex amylo accompanies a glusosuria e saccharo. In the first instance it is scarcely likely that true diabetes will develop in the course of time, while in the latter this is at least possible.

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[1] "Psoriasis und Glycosurie," Berlin. klin. Woch., 1900, No. 2.
[2] Wien. med. Presse, 1895, vol. xxxvi.
[3] "La glucosuria da diuretina," Il Policlinico. 1900, No. 5.

Aside from the digestive form of glucosuria which has just been considered, and which is produced artificially, an idiopathic transitory form is also known to occur. A *transitory glucosuria,* apparently of central origin, is thus noted in connection with lesions affecting the central as well as the peripheral nervous system, such as tumors and hemorrhages at the base of the brain, lesions of the floor of the fourth ventricle, cerebral and spinal meningitis, concussion of the brain, fracture of the cervical vertebræ, tetanus, sciatica; following epileptic, hystero-epileptic, and apoplectic seizures, mental shock produced by railroad accidents (traumatic neuroses), etc.; mental strain and worry, fatigue, and anxiety. Glucosuria following epileptic and apoplectic attacks, however, does not appear to be so common as is generally believed. v. Jaksch was unable to demonstrate the presence of sugar in 50 recent cases of hemiplegia, and in a large number of cases of epilepsy, with urines voided within the first few hours following the seizure, I have reached only negative results.

In Basedow's disease transitory glucosuria may also occur, and it is well established that a relation may exist between the disease in question and the complex of symptoms designated as diabetes mellitus.[2]

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[1] E. Külz, Beiträge zur Pathol. u. Therap. d. Diabetes, Marburg, 1874, vol. i, p. 110.

[2] Dumontpallier, "Goiter exophthalmique et glycosurie," Compt.-rend. de la soc. de biol., 1867. O'Neill, "Exophthalmic Goitre and Diabetes occurring in the Same Person," Lancet, 1878, pt. i, p. 9. S. Bettmann, Münch. med. Woch., 1896, vol. xliii, Nos. 49 and 50. E. Grawitz, Fortsch. d. Med., 1897, vol. xv. K. Osterwald, Inaug. Diss., Göttingen, 1898. H. Stern, Jour. Amer. Med. Assoc., 1902, vol. xxxix, p. 972.

Siegmund noted a transitory glucosuria in 52.38 per cent. of general paretics, in 7.4 per cent. of epileptics, and in 3.77 per cent. of dementia cases, while it was not observed in other mental diseases. In reference to the postepileptic glucosuria which has been noted by some of the older observers more especially, an analysis of their work has led me to the conclusion that their inferences were scarcely justifiable, as a wholly satisfactory proof of the presence of sugar has not been furnished.[1]

In cases of cholelithiasis, contrary to what has been maintained by one or two observers, glucosuria is unusual.

It is well known that Claude Bernard experimentally produced a transitory glucosuria by puncturing a certain spot in the floor of the fourth ventricle, the supposed origin of the hepatic vasomotor nerves, and it is not improbable that this neurotic form of glucosuria is due to some direct or reflex influence affecting that portion of the medulla.

The transitory glucosuria occasionally observed in acute febrile diseases, such as typhoid fever, scarlatina, measles, cholera, diphtheria, influenza, and especially malaria, particularly during convalescence, may possibly be referable to the action of specific toxins upon this centre. Seegen reports 5 cases of malaria with "diabetes" in which *both conditions* disappeared under the administration of quinine. In diphtheria glucosuria appears to be of common occurrence. Binet thus obtained a positive result in 29 cases out of 70; 27 times in severe infections out of 38, and twice in mild cases out of 32. I have personally found a transitory glucosuria in 4 cases out of 32; the infection in these was of moderate severity. Hibbard and Morrissey arrived at similar results.[2]

A glucosuria of toxic origin has been noted in cases of poisoning with curare, chloral hydrate, sulphuric acid, arsenic, alcohol, carbon monoxide, morphine, etc., and even after simple transfusion of normal salt solution into the blood. Phloridzin, a glucoside obtained from the bark of the root of the apple tree, will likewise cause sugar to appear in the urine. The glucosuria thus produced is, however, only temporary, and ceases upon withdrawal of the drug.[3] Of interest is the glucosuria which occasionally follows the administration of thyroid extract or of iodothyrin, as there is evidence to show that in such cases a special predisposition to glucosuria exists. When carried to an extreme degree true diabetes may develop, which subsequently cannot be arrested by withdrawal of the substance.[4]

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[1] See, also, Araki., Zeit. f. phys. Chem., vol. xv, p. 363.

[2] "Glycosurie in Diphtheria," Jour. Exper Med., vol. iv, p. 137.

[3] Zuntz, "Zur Kenntniss d. Phloridzindiabetes," Du Bois' Archiv, 1895, p. 570.

[4] H. Strauss, "Neurogene and thyreogene Glucosurie," Deutsch. med. Woch., 1897, Nos. 19 and 20.

The occurrence of a transitory glucosuria under the conditions bove mentioned, and which may be met with in almost any disease, oreover, while interesting from a theoretical standpoint, must in e majority of instances be regarded as a medical curiosity only, nd it is but rarely possible to draw either diagnostic, prognostic, or erapeutic conclusions from its existence.

A *persistent form of glucosuria* is noted in connection with certain sions of the brain, especially those affecting the floor of the fourth entricle, and is at times of considerable value in diagnosis. This also observed after removal of the thyroid gland, and in cases in hich thyroid extract has been administered in unduly large amount.

A continuous elimination of sugar, however, is noted principally the complex of symptoms to which the term *diabetes mellitus* has een applied.

Diabetes mellitus is essentially a persistent form of glucosuria sociated with the occurrence of a more or less intense polyuria and greatly increased elimination of all the metabolic products normally und in the urine, with the exception of uric acid, which is usually resent in diminished amount. In the more advanced cases acetouria, lipuria, and lipaciduria may also exist. Diabetes, however, is ot a persistent form of glucosuria in an absolute sense of the word, s periods may occur in the course of the disease when glucose is mporarily absent.

The quantity of sugar excreted may be very large, and 180 to 360 rams pro die are amounts which may be frequently observed. his quantity may diminsh to zero under various conditions, such s the occurrence of intercurrent diseases, but often also without any pparent cause, and not infrequently in the condition which has been rmed diabetic coma. Cases are also observed in which from beginning to end mere traces are eliminated, the total amount of sugar ot exceeding a few grams, while the course of the disease rapidly nds toward a fatal termination, *so that the severity of the pathological rocess cannot be measured by the amount of sugar eliminated.*

At the same time it should be remembered that diabetes cannot e excluded by one or even more negative urinary examinations, and e value of repeating such examinations three or four hours after e exhibition of 100 grams of glucose, as indicated, cannot be too rongly urged.

Clinicians are in the habit of determining the severity of a case, a certain extent at least, from the condition of the urine under a diet ee from starches and sugars, and generally regard those cases as the ore serious in which the glucosuria does not disappear under a diet f this character, while a more favorable prognosis is given if the gar disappears. It should be remembered, however, that there re numerous exceptions to this rule, and that a light case—*i. e.*, ne in which the sugar disappears under appropriate dietetic treatment—may suddenly exhibit symptoms seen only in the most severe forms, or succumb to one of the numerous intercurrent maladies, while apparently severe cases may assume the more benign type.

It may not be out of place in this connection to say a few words regarding the specific gravity of the urine. While usually very high, varying between 1.030 and 1.060, as pointed out in the chapter on Specific Gravity, comparatively low figures are noted at times, such as 1.012, corresponding to a quantity of urine not exceeding 1000 c.c., and implying, of course, a diminished elimination of solids. This is especially marked in those cases described by Hirschfeld,[1] in which, as pointed out in the chapter on Urea, the resorption of nitrogenous material from the digestive tract is below the normal. Polyuria, a fairly constant symptom of the more common types of diabetes mellitus, is much less pronounced in Hirschfeld's form, and may be altogether absent, although it is true that this may occur in ordinary diabetes also.

The simultaneous occurrence of glucosuria, acetonuria, lipuria, and lipaciduria (which see) is probably always indicative of true diabetes.

It is, of course, impossible to enter here into a detailed consideration of the origin of diabetes. Suffice it to say that a persistent glucosuria, aside from nervous influences, may be referable, on the one hand, to an inability on the part of the liver to transform into glycogen all of the sugar which is carried to this organ; or, on the other hand, to an inability on the part of the muscular system of the body to utilize all the sugar sent to it. Accordingly, we may distinguish between a *hepatogenic* and a *myogenic diabetes.*

Within recent years it has been shown that pancreatic disease is frequently associated with diabetes, and while the number of cases in which no pancreatic lesions are discovered is still too large to warrant the conclusion that disease of this organ is invariably associated with glucosuria, it still must be admitted that lesions of the pancreas are the more frequently met with in diabetes the more carefully the organ is examined. So much appears to be certain, that diabetes *may* be produced by pancreatic disease. As to the manner, however, in which such a result can occur we are in ignorance. In this connection it is interesting to note that, according to Opie, disease of the areas of Langerhans more especially is associated with the clinical picture of diabetes, while lesions affecting the secreting portion of the gland only do not influence the carbohydrate metabolism.[2] These observations of Opie have been largely confirmed by other observers.

In cancer of the pancreas glucosuria only occurs in a small per-ge of cases—3 of 21, as reported by Pearce.[1] In 1 of these was a true cancer diabetes with involvement of the islands, in a second case the glucosuria was intermittent without manihanges.

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[1] "Ueber eine neue klinische Form d. Diabetes," Zeit. f. klin. Med., vol. xix, pp 294 and 325.

[2] Opie, Jour. Exper. Med., 1901, vol. v, p. 527.

schfeld pointed out that while in the majority of diabetic patients roteid food is quite satisfactorily utilized, the assimilation of nd albumins is much below normal in others, and particularly the pancreatic cases. (See also Urea.) Observations in this ion are as yet very scanty, so that a definite opinion cannot be ssed regarding the utility in diagnosis of investigations similar se of Hirschfeld.

ether or not a renal and a thyroigenic diabetes exists, as has suggested, remains an open question.[2] That Basedow's disease e associated with diabetes mellitus I have already pointed out.

ts for Sugar.—The tests for sugar usually employed in the l laboratory depend upon the following properties of sugar:

In the presence of alkalies it acts as a reducing agent upon n metallic oxides, such as those of copper and bismuth (Feh-, Trommer's, Böttger's, and Nylander's tests).

In the presence of yeast (Saccharomyces cerevisiæ) it under-ermentation, with the formation of ethyl alcohol, carbonic acid, ic acid, glycerin, amyl alcohol, etc. (fermentation test).

With phenylhydrazin sugar forms an insoluble crystalline com-d—phenylglucosazone.

Solutions of glucose turn the plane of polarized light to the from which property glucose has also received the name *se.*

every case the urine should first be tested for the presence of in, which should be removed by boiling.

mmer's Test.[3]—A few cubic centimeters of urine are strongly nized with sodium hydrate solution, and treated with a 5 per solution of cupric sulphate, added drop by drop, until the c oxide formed is no longer dissolved. The mixture is care-heated, when in the presence of sugar a yellow precipitate of us hydroxide is formed, which gradually settles to the bottom sediment of red cuprous oxide.

is important to note that while sugar, unless present in mere , can readily be detected in this manner, other substances are

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ner. Jour. Med. Sci., Sept., 1904, p. 478.

abetes: J. Seegen, Die Zuckerbildung im Thierkörper, Berlin, 1890, p. 260. rden, Pathol. d. Stoffwechsels, Berlin, 1893. Seegen, "Ueber d. Zucker-d. Blutes von Diabetikern," Wien. med. Woch., 1886, Nos. 47 and 48. Pavy, "Ueber die Behandlung von Diabetes mellitus," Verhandl. d. X. at. Med. Congr., 1891, ii, Abt. 5, p. 80. P. F. Richter, "Nierendiabetes," h. med. Woch., 1899, p. 840.

nal. d. Chem. u. Pharm., 1841, vol. xxxix, p. 361.

or may be present in the urine, such as uric acid, kreatin and kreatinin, allantoin, nucleo-albumin, milk sugar, pyrocatechin, hydroquinone, and bile pigment, which likewise reduce cupric oxide. Following the ingestion of benzoic acid, salicylic acid, glycerin, chloral, sulphonal, etc., reducing substances also appear. These may generally be disregarded, it is true, if care is taken *not to boil* the urine after the addition of the cupric sulphate, as the precipitation of cuprous oxide in the presence of sugar takes place before this point is reached. Unfortunately, however, the test when thus applied yields negative results, or results which are doubtful, if traces only are present, so that it cannot be utilized, as a rule, in the study of transitory or digestive glucosuria.

Fehling's Test.[1]—This is a modification of the test just described, and can be recommended only with the same restrictions.

Two solutions are employed, which must be kept in separate bottles, the one containing 34.64 grams of crystallized cupric sulphate, dissolved in 500 c.c. of distilled water, and the other 173 grams of potassium and sodium tartrate and 50 to 60 grams of potassium hydrate, dissolved in an equal volume of water. Equal parts of the two solutions, mixed in a test-tube and diluted with four times as much water, are boiled, when a small amount of urine is added. In the presence of sugar a precipitate of the yellow hydroxide of copper or of red cuprous oxide will be produced; but *care should be taken only to warm, and not to boil the solution after the addition of the urine.*

Not infrequently it will be observed that upon standing, when no precipitation has occurred previously, the blue color of the mixture changes to an emerald green, while the solution at the same time becomes turbid. Such a phenomenon should not be referred to the presence of sugar, as it is in all probability due to the action of other reducing substances, such as those mentioned above.

Böettger's Test with Nylander's Modification.[2]—A few cubic centimeters of urine are treated with *Almén's solution* in the proportion of 11 to 1. This is prepared by dissolving 4 grams of potassium and sodium tartrate, 2 grams of bismuth subnitrate, and 10 grams of sodium hydrate in 90 c.c. of water, heating the solution to the boiling point and filtering upon cooling, when it should be kept in a colored glass bottle. The mixture of urine and Almén's fluid is thoroughly boiled, when in the presence of sugar a grayish, dark-brown, and finally a black precipitate, consisting of bismuthous oxide or of metallic bismuth, is obtained. Albumin, if present, must first be removed, as, owing to the sulphur contained in the albuminous molecule, alkaline sulphides would be formed upon boiling, and, acting upon the bismuth, give rise to the formation of black bismuth sulphide, which might be mistaken for metallic bismuth. Rhubarb pigment, as well as melanin and melanogen (which see), and free hydrogen sulphide must also be absent, as misleading results will otherwise be obtained.

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[1] Annal. d. Chem. u. Pharm., 1849, vol. lxxii. p. 106.

[2] Zeit. f. physiol. Chem., 1883, vol. viii, p. 175.

Nylander's test, as well as that of Trommer and Fehling, is, however, not without objections, as a partial reduction of the bismuth subnitrate may be produced by other substances, such as kairin, tincture of eucalyptus, turpentine, and large doses of quinine.

Fermentation Test.[1]—This is based upon the fermentative decomposition of sugar with the formation of carbon dioxide and alcohol.

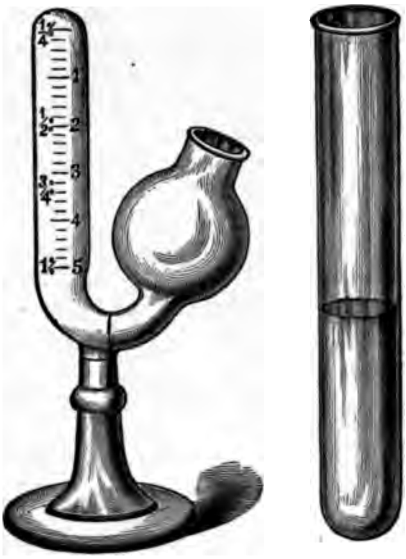

FIG. 148.—Einhorn's saccharimeter.

It should be resorted to in all doubtful cases. The test is now almost always carried out in special fermentation tubes, such as those of Einhorn (Fig. 148) and Lohnstein (Fig. 149). To this end a small piece of compressed yeast (a fair sized pill) is broken up in a test-tubeful of urine. It is better to do this with a glass rod than by shaking. The fermentation tube is filled with this mixture, care being taken that no bubbles of air remain at the top. A little mercury is poured in, so as to occlude the lower bend, after which the tube is kept at a temperature of 30° to 38° C. for twenty to twenty-two hours. At the end of this time it is inspected to see whether any gas has been formed. In the case of sugar urines it can readily be proven that the gas is carbon dioxide by introducing some caustic alkai into the tube, when the gas is absorbed.

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[1] E. Salkowski, Berlin klin. Woch., 1905, p. 48

In every case it is necessary to make a control test with normal urine of approximately the same concentration, as the common commercial yeast always develops a little carbon dioxide by itself. A little bubble is thus usually seen. But the same may occur from the liberation of gas which may be present in absorption, when the test is kept at the temperature indicated. Unless traces of sugar (less than $\frac{1}{10}$ per cent.) be present no difficulty will result from this fact, as the volume of gas in the sugar urine will exceed that of the control. But when smaller quantities are present some doubt may arise. In that case an attempt must be made to increase the volume of gas by heating, when the sugar urine owing to the presence of carbon dioxide will show a larger bubble of gas than the control. This may be done on a boiling water bath by placing both fermentation tubes in a large beaker filled with water such that the tops of the tubes are just covered, and heating for half an hour.

If a positive result is obtained with the fermentation test the presence of a fermentable sugar is proven; the question whether this is dextrose or levulose, which alone enter into consideration in disease, is practically unimportant. Should blood, pus, albumin, or albumose be present, these should first be removed.

Rarely it will happen that the urine undergoes ammoniacal decomposition in the tubes; if it does occur the examination should be repeated.

Phenylhydrazin Test.[1]—As originally proposed by v. Jaksch, the test is conducted as follows: 6 to 8 c.c. of urine are treated with 0.4 to 0.5 gram of phenylhydrazin hydrochlorate and 1 gram of sodium acetate, and warmed until the salts have been dissolved, a little water being added if necessary. The tube is placed in boiling water for twenty to thirty minutes, and then transferred to a beaker filled with cold water. If sugar is present in moderate amounts, a bright-yellow, crystalline deposit will at once be thrown down and partly adhere to the sides of the tube. But even in the presence of mere traces a careful microscopic examination will reveal the presence of crystals of phenylglucosazone. These are seen singly or arranged in bundles and sheaves composed of delicate, bright-yellow needles which are insoluble in water.

Still more convenient is the following modification of the test, as suggested by Cipollina:[2] 5 drops of pure phenylhydrazin, 0.5 c.c. of glacial acetic acid, or 1 c.c. of 50 per cent. acetic acid are placed in a test-tube together with 4 c.c. of urine. The mixture is boiled for about one minute over a small flame, while shaking so as to avoid bumping as much as possible; 4 or 5 drops of sodium hydrate solution (specific gravity 1.16) are added, but the solution must remain acid; the boiling is continued for a few seconds and the mixture then allowed to cool. The rapidity with which the glucosazone crystals separate out depends somewhat upon the specific gravity of the urine. If this is low they form in a few minutes, even though the amount of sugar does not exceed 0.05 per cent. If, on the other hand, the specific gravity is high, yellow balls and thornapple forms result, while typical rosettes develop only after twenty to thirty minutes, and at times one is even then left in doubt as to the result. If the urine contains more than 0.2 per cent. of sugar, however, even though the specific gravity be high, the formation of typical crystals occurs within a few minutes. If with this modification no crystals are obtained at the expiration of an hour, we may infer that no sugar is present.

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[1] v. Jaksch, Zeit. f. klin. Med., 1886, vol. xi, p. 20.

[2] Deutsch. med. Woch., 1901, vol. xxvii, p. 334.

This test, properly applied, is undoubtedly not only the most delicate, but at the same time the most reliable, as no other substances which may be present in the urine, excepting maltose and certain pentoses, will give rise to the formation of an osazone. Hence, whenever doubt is felt as to the nature of a substance reacting in a positive manner with the reagents described above, recourse should be had to this test. It has been stated that maltose forms an exception; this, however, will never become embarrassing, as the microscopic appearance of the maltosazone crystals differs from that of the phenylglucosazone. The melting point of phenylglucosazone (205° C.), moreover, is about 15° C. higher than that of the maltosazone—190° to 191° C. To determine this point, it is necessary to filter off the osazone, and, after washing with water, to dissolve it upon a filter by means of a little hot alcohol. From this alcoholic solution it is reprecipitated by water, when it may be collected and dried over sulphuric acid. The melting point is then determined according to the usual methods.

The pentosazones also can be readily distinguished from glucosazone by their melting points (which see).

The amount of lactose which may be found in the urine is far too small to give rise to the formation of an osazone when the test is directly applied to the urine.

With the conjugate glucuronates phenylhydrazin also combines to form crystalline compounds, but these may likewise be distinguished by their melting points and the form of the crystals. Such compounds, moreover, are usually not present in amounts sufficient to give rise to confusion. (See Glucuronic Acid.)

Polarimetric Test.—Glucose turns the plane of polarized light to the right, but the same may be said of maltose, the degree of polarization of which is even more marked, so that it may be impossible to state in a given case whether such rotation is referable to a large quantity of glucose or to a smaller quantity of maltose. The latter substance, however, occurs in the urine but rarely, and may be recognized not only by the microscopic appearance of its osazone, but also by the fact that its power of reduction is increased in the presence of sulphuric acid and by the application of heat.

An error which may further arise with the employment of the polarimetric method is referable to the fact that if glucose is present in only small amounts, while the urine contains large quantities of β-oxybutyric acid, the latter turning the plane of polarized light to the left, it may happen that the rotation in this direction will neutralize or even counterbalance any rotation to the right, which may be due to glucose. In such cases, however, the urine will react in a positive manner with the other reagents described, and the fermented urine will, moreover, turn the plane of polarization still more strongly to the left, indicating the presence of a dextrorotatory substance, and in all probability of glucose.

The delicacy of this method varies with the instrument employed; the figures given below were obtained with the apparatus of Lippich, which yields the best results. (For a description of this method see the Quantitative Estimation of Sugar by Means of the Polarimeter.)

Table showing the Delicacy of the Tests described.

Trommer's test	0.0025	per cent.
Fehling's test	0.0008	"
Nylander's test	0.025	"
Fermentation test	0.1–0.05	"
Phenylhydrazin test	0.025–0.05	"
Polarimetric test	0.025–0.05	"

Table showing the Behavior of the Various Forms of Sugar which may occur in the Urine toward the Tests described.

	Trommer's, viz., Fehling's test.	Nylander's test.	Fermentation test.	Phenylhydrazin test.	Polarimetric test.
Glucose.	Positive reaction.	Positive reaction.	Positive reaction.	Positive reaction; melting point 205° C.	Rotation toward the right.
Levulose.	Positive reaction.	Positive reaction.	Positive reaction.	Same osazone obtained as with glucose, only more rapidly.	Rotation toward the left.
Maltose.	Positive reaction.	Positive reaction.	Positive reaction.	A maltosazone is formed; melting point 190°-191° C.	Rotation toward the right.
Lactose.	Positive reaction.	Positive reaction.	No reaction or only a very faint one.	No reaction in the concentration in which it may occur in the urine; melting point 200° C.	Rotation toward the right; increased by boiling with a 2.5 per cent. solution of sulphuric acid.
Laiose	Positive reaction on boiling only; 1.2-1.8 per cent. more is obtained than by the polarimeter.	Positive reaction.	No reaction.	With phenylhydrazin a yellowish-brown, non-crystallizable oil is obtained.	No reaction, or rotation toward the left.

Clinically, it is unimportant to search for minute traces of sugar, such as may be found in every normal urine, and the reader is referred to special works on physiological chemistry for a consideration of the methods generally employed. (See method of Baumann and v. Udranszky.)

Quantitative Estimation of Sugar.—The methods used in the quantitative estimation of sugar are essentially based upon the qualitative tests described.

Fehling's Method.—The Fehling solution (see above: qualitative tests) must be accurately standardized as follows: 0.2375 gram of pure crystallized cane sugar, dried at 100° C., is dissolved in 40 c.c. of distilled water, to which 22 drops of a 10 per cent. solution of sulphuric acid have been added. This solution is kept on the boiling water bath for an hour, when it is allowed to cool and diluted to 100 c.c. with distilled water; 20 c.c. of this solution will then contain exactly 0.05 gram of glucose, corresponding to 10 c.c. of Fehling's solution, if this is of the required strength. If too strong, so that 21 c.c. of the sugar solution, for example, are required to obtain a complete reduction of the copper, the strength of Fehling's solution may be determined according to the equation: $20 : 0.05 :: 21 : x$; and $x = 0.0525$. If too weak, on the other hand, so that 19 c.c., for example, are required, its strength is similarly determined: $20 : 0.05 :: 19 : x$; and $x = 0.0475$.

If the solution is of the theoretically required strength 10 c.c. will correspond to 0.05 gram of glucose.

If then urine is added to this quantity until complete reduction has taken place, the amount of sugar in a given specimen of urine can be calculated according to the following equation:

$$y : 0.05 :: 100 : x; \text{ and } x = \frac{5}{y},$$

in which y indicates the number of cubic centimeters of urine required to reduce the 10 c.c. of Fehling's solution, and x the amount of sugar contained in 100 c.c. of urine.

As the best results are obtained if from 5 to 10 c.c. of urine are used in one titration, it is often necessary to dilute the urine to this end; in the determination of this point the specific gravity may serve as a guide. As a general rule, urines of a specific gravity of 1.030 should be diluted five times, and if the density is still higher ten times. Albumin, if present, must first be removed by boiling: 10 c.c. of Fehling's solution diluted with 40 c.c. of water are placed in a porcelain dish and boiled. While boiling, the diluted urine is added from a burette, 0.5 c.c. at a time, when, as a rule, the precipitated cuprous oxide will settle, so that the white sides of the dish may be seen through the blue field. In my experience it is very helpful to boil the mixture for a few moments after every addition of urine and to stir thoroughly each time with a rubber-tipped rod. In this way the precipitate is prevented from forming a coating on the vessel and settles down more readily. As the end point is reached every trace of blue has disappeared and the liquid has a faint yellowish tinge owing to beginning caramelization of the excess of sugar by the caustic alkali.

If any doubt should arise whether the end point has been reached, tiny droplets of the mixture should be placed upon ferrocyanide paper (prepared by soaking filter paper in a moderately dilute solution of potassium ferrocyanide). If unreduced copper is still present a brown color results. The result is regarded as positive only, if the brown develops at once. If it occurs only after several seconds the final point has been reached or passed.

Prolonged boiling always brings some copper into solution again. It is hence advisable to make two examinations always, the one approximately only, and the second as the final one.

The calculation is then made as indicated above.

EXAMPLE.—The volume of urine for twenty-four hours was 4000 c.c. It was diluted five times; 6 c.c. of the diluted urine brought about the complete reduction of 10 c.c. of Fehling's solution; the 6 c.c. hence contained 0.05 gram of sugar; 100 c.c., accordingly, contained 0.833 gram. As the urine had been diluted five times this figure must be multiplied by 5 = 4.165, which is the percentage for the native urine. The amount for the twenty-four hours was hence 4.165 = 40 (hundreds) × 166.6 grams.

Gerrard and Allan's Method (Modified by Rudisch and Celler).[1] —To obviate some of the difficulties which attach to Fehling's method Rudisch and Celler have recently suggested the following modification of Gerrard and Allan's method:

"To four parts by volume of a 50 per cent. solution of potassium sulphocyanate, chemically pure, is added one part by volume of a mixture of equal parts of Fehling's copper sulphate and alkaline solutions. 25 c.c. of this solution are placed in a porcelain dish, and the urine to be tested added drop by drop from a burette until the blue color of the copper entirely disappears. Throughout the titration the solution should be slowly boiled and constantly stirred with a glass rod. The end reaction is extremely sharp, the fluid becoming colorless or assuming a faint-yellow tinge. The advantages of this method are: (1) only one titration is necessary, as potassium sulphocyanate does not decolorize the copper solution; (2) potassium sulphocyanate is not poisonous; (3) as the mixture is stable a considerable quantity may be made to be kept as 'stock.' Such a 'stock' solution was found to be unaffected after four months' exposure to heat and sunlight.

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[1] Jour. Amer. Med. Assoc., January 26, 1907.

"With aqueous solutions of glucose ranging from 0.25 to 6 per cent. the results obtained with this method and with the polariscope are identical. With diabetic urines, however, variations of from 0.03 to 0.25 per cent. are occasionally found—differences that are too small to be of clinical significance. These variations are explicable on two grounds. First, substances other than glucose (creatinin, uric acid, glucuronic acid) reduce copper and give too high a reading with Fehling's solution; secondly, levorotating substances (albumin, levulose, β-oxybutyric acid) may coexist with the glucose in the urine, giving too low a percentage with the polariscope. To estimate properly the quantity of dextrose in any given specimen, therefore, it is necessary to make determinations both with the copper solution and with the polariscope. Should the former indicate a higher percentage than the latter, levulose should be suspected and tested for with the Seliwanoff resorcin-hydrochloric acid method. In the absence of levulose the most probable disturbing factor is β-oxybutyric acid, as albumin and other levorotators are precipitated when the urine is cleared with lead acetate for the polariscope.

"Although with undiluted urines containing large amounts of dextrose satisfactory results have been obtained with this method, the extreme care necessary in titrating under these conditions makes it advisable to dilute such urine from five to ten times. It is preferable to examine specimens when fresh, but, should it become necessary to employ preservatives, toluol, salicylic acid, or carbolic acid may be added in small quantities without markedly interfering with the reaction. Chloroform, on the other hand, must be avoided, as even in minute traces its presence vitiates the test.

"In calculating the percentage of sugar by the above method it must be remembered that the titre of the copper solution is unchanged by the addition of the solution of potassium sulphocyanate, and that the mixture represents Fehling's solution diluted five times. Each c.c. of the reagent will therefore be reduced by 1 mgm. of sugar.

"For example, if for the decolorization of 25 c.c. of the mixture, equivalent to 25 mgm. of sugar, 1.2 c.c. of undiluted urine are used, then 1 c.c. of the urine will decolorize 25 divided by 1.2=20.8 c.c. of the reagent, equivalent to 28.8 mgm. of sugar, or 2.08 per cent.

"If 0.75 c.c. of urine decolorize 25 c.c. of the reagent, 1 c.c. will decolorize 25 divided by 0.75=33.3 c.c. of reagent, equivalent to 33.3 mgm. of sugar, or 3.33 per cent."

Differential Density Method.[1]—This method is very useful in clinical work, and should be preferred to the more uncertain titration with Fehling's solution.

The specific gravity is accurately ascertained by means of a pyknometer, or a hydrometer graduated to the fourth decimal and provided with a thermometer indicating tenths of a degree. The temperature at which the specific gravity is taken should be that for which the hydrometer has been constructed, the urine being heated or cooled to the desired degree; 100 to 200 c.c. are set aside in a flask, loosely stoppered after the addition of a small piece of yeast, which should be finely divided. After twenty-four hours if but little sugar is present, or forty-eight hours if there is much, the specific gravity is again determined under the precautions given, after having filtered the urine. The difference in the specific gravity is multiplied by 230, an empirical factor which has been found by dividing the amount of sugar ascertained by titration or polarization with the difference in the density of the urine after fermentation. The result indicates the percentage of sugar. Evaporation must be guarded against by using a bulbed safety tube containing some alkaline solution.

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[1] Roberts, Lancet, 1862, i, p. 21. Worm-Müller, Pflüger's Archiv, 1884, vol. xxxiii, p. 211, and 1885, vol. xxxvii, p. 479.

The process may be hastened if to each 100 c.c. of urine 2 grams of potassium and sodium tartrate and 2 grams of diacid-sodium phosphate are added, with 10 grams of compressed yeast, and the mixture is kept at a temperature of from 30° to 34° C. If but little sugar is present, two or three hours will be sufficient. That portion of the urine of which the specific gravity is determined before fermentation should really be treated in the same manner. It will suffice, however, to add 0.022 to the specific gravity found, to make up for the increase that would otherwise be observed in the second specimen owing to the addition of the salts.

In every case the urine must be perfectly fresh, as fermentation generally begins spontaneously, even after standing a short time.

Einhorn's Method. — This will answer very well for ordinary purposes. Two especially constructed and graduated saccharimetric tubes (see Fig. 148) are used, one of which is filled with a mixture of the suspected urine and yeast, and the other with normal urine and yeast, as a control. The examination in general is conducted as described before. (See Qualitative Tests for Sugar.)

Lohnstein's Method. — A very convenient modification of Einhorn's instrument, and one furnishing more accurate results, has been introduced by Lohnstein.[1] As will be seen from the accompanying figure (Fig. 149), this is essentially a U-tube open at both ends. The longer limb is closed during the process of fermentation by a ground-glass stopper. This stopper is provided with an air-hole, to which a similar hole corresponds in the drawn-out portion of the tube. The apparatus is filled with the urine to be examined, through the bulb *A*, while the two air-holes at *B* are in communication. Care should be had that the liquid stands exactly at the mark 0. The stopper is then turned so that all communication between the air and the urine is cut off. A little mercury is finally poured into the saccharimeter, when the instrument is maintained at a temperature of about 30° to 38° C. After twelve hours the percentage of sugar is read off directly.

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[1] "Ein neues Gährungssaccharometer," Berlin. klin. Woch., 1898, p. 866.

Precautions: 1. As every urine contains traces of free carbon dioxide, it is well to remove this by boiling if we have reason to suppose that only a small amount of sugar is present. Before adding the yeast the urine is, of course, cooled to the surrounding temperature.

2. As the instrument yields satisfactory results only if the urine contains less than 1 per cent. of sugar, it is necessary to dilute it with water when more is present. The specific gravity may here serve as an index; urines of a specific gravity up to 1.018 are examined directly; from 1.018 to 1.022 they are diluted twice, from 1.022 to 1.028 five times, and those above 1.028 ten times.

3. A test-tube, provided with the necessary marks to indicate the degree of dilution of the urine, accompanies the instrument. In every case a globule of yeast, approximately 6 to 8 mm. in diameter, is added to the urine and shaken in the tube

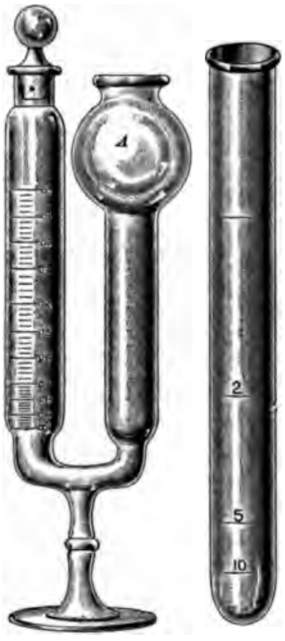

Fig. 149.—Lohnstein's saccharimeter.

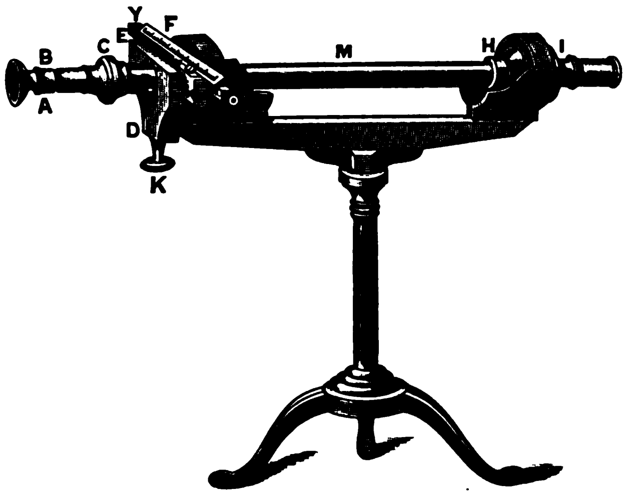

Fig. 150.—Soleil-Ventzke's saccharimeter.

until an even suspension has been reached.[1] (See also Qualitative Tests for Sugar.)

Polarimetric Method. — For this purpose the saccharimeter of Soleil-Ventzke is very convenient (Fig. 150). This consists essentially of a Nicol prism, *A*, which may be rotated about the axis of the apparatus; a second Nicol prism, at *D*; vertically placed compensating prisms, consisting of dextrorotatory quartz, at *E*, which may be moved horizontally by means of a rack-and-pinion adjustment, turned by a milled head at *K*, so that light can pass through a thicker or thinner layer of the dextrorotatory quartz. At *F* is a plate of levorotatory quartz cut perpendicularly to the optical axis, and covering the entire field of vision; at *H* biquartz plates of Soleil, and at *I* an Iceland-spar crystal; *B* and *C* represent a small telescope, by means of which the biquartz plates can be accurately focussed. When the compensation prisms of this apparatus are in a certain position the levorotation of the plate *F* will be exactly compensated, and the two halves of the field of vision present the same color, while the zero of the scale *X* will coincide with the zero of the vernier *Y*, arranged on the upper surface of the compensators. Any change in this position produced by turning the screw *K* will cause the appearance of a different color in each half of the field of vision. If now, with a zero position, an optically active dextrorotatory or levorotatory substance is interposed, the color of each half of the field of vision will become altered, but may be equalized again by changing the position of the compensators, the degree of change necessary to produce this result constituting an index of the power of rotation of the solution interposed in the tube *M*.

Soleil-Ventzke's apparatus is constructed in such a manner that if a solution of glucose is employed, the length of the tube *M* being 10 cm., every entire line of division on the scale will indicate 1 per cent. of sugar.

The tube of the saccharimeter should be carefully washed out with distilled water, and at least once or twice with the filtered urine, when it is placed on end upon a flat surface and filled with the urine, so that this forms a convex cup at the end. The glass plate is now carefully adjusted, so as to guard against the admission of bubbles of air. The metallic cap is placed in position, care being taken to avoid undue pressure. The examinations are made in a dark room; an ordinary lamp is used, and several readings are taken, until the differences do not amount to more than 0.1 or 0.2 per cent. The tubes should be thoroughly cleansed *immediately* after the experiment.

In every case the filtered urine should be free from albumin, and, if markedly colored, should be previously treated with neutral lead acetate in substance and filtered.

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[1] Lohnstein's saccharimeter may be procured from R. Kaltmeyer & Co., Oranjenburger Str. 45, Berlin.

If it is only desired to demonstrate the presence of sugar, the compensators are first brought to the zero position. If now, upon interposition of the tube filled with urine, a difference in the color of the two halves of the field of vision is noted, the presence of an optically active substance in the urine may be assumed; and if the deviation is at the same time to the right, the presence of glucose is rendered highly probable, while a deviation to the left will generally be referable to levulose or β-oxybutyric acid. Indican, peptones (albumoses), cholesterin, and certain alkaloids, it is true, also turn the plane of polarization to the left; but as a rule these substances need not be considered, as cholesterin occurs but rarely, and indican is usually present in only small amounts in diabetic urines. Albumoses, if present, must first be removed. Lactose and maltose, which also turn the plane of polarization to the right, may be distinguished from each other and from glucose by the phenylhydrazin test. Levulose turns the plane of polarization to the left. Oxybutyric acid is practically always associated with the presence of glucose, and may be recognized by allowing the urine to undergo fermentation, when the filtered urine will become distinctly levorotatory.

Lactose.—Lactose[1] is a normal constituent of the urine during the last weeks of pregnancy and the first weeks following childbirth. The antepartum lactosuria usually amounts to about 1 gram pro liter, but may reach 2 grams and rarely even higher figures. The postpartum lactosuria is more marked. It reaches its maximum between the third and fifth day after labor, the amount varying between 1 and 8 grams pro liter.

After lactation is once well established lactose is not usually found in the urine, but it may occur if for any reason milk stasis occurs.

Occasionally lactosuria is accompanied by a mild grade of glucosuria.

An alimentary lactosuria may follow the ingestion of 60 grams of lactose, though as a general rule 120 grams may be regarded as the limit of tolerance.

The presence of lactose may be inferred if a positive result is obtained with Trommer's and Nylander's tests, while the phenylhydrazin and fermentation tests give negative results. An osazone may, however, be obtained from the *isolated* substance.

Levulose.[2]—An alimentary levulosuria occurs after the ingestion of more than 140 to 160 grams of sugar. In severe cases of diabetes levulose may be found in the urine together with glucose, even though the food contains neither levulose nor other carbohydrates. Such an occurrence is regarded as a grave omen.

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[1] De Sinety, Maly's Jahresber., 1874, vol. iii, p. 134. Hempel, Arch. f. Gynäk., 1875, vol. viii, p. 312. Ney, ibid., 1889, vol. xxxv, p. 239. F. Hofmeister, "Ueber Laktosurie," Zeit. f. physiol. Chem., 1877, vol. i, p. 101 (lit.). F. A Lemaire, ibid., 1896, vol. xxi, p. 442. Commandeur and Porcher, Arch. gén. de méd., 1904, pp. 2241 to 2305.

[2] Seegen, Centralbl. f. d. med. Wiss., 1884, vol. xxii, p 753. H. Rosin and L. Labaud, Zeit. f. klin. Med., vol. xlvii, Heft 1 u. 2.

Spontaneous levulosuria unaccompanied by glucosuria has also been described. Such urines show a deviation to the left or none at all, while the other tests for sugar indicate the presence of a reducing substance.

Maltose.—Maltose, together with glucose, was first found in the urine of a patient supposedly the subject of pancreatic disease, associated with an acholic condition of the stools. Since that time it has been repeatedly observed in diabetic patients. In one case the amount was 27.8 grams pro liter. Similar results have been obtained in dogs after extirpation of the pancreas.[1] Its recognition is practically dependent upon the formation of its osazone and a determination of the melting point of the latter. Such urines, moreover, show a larger percentage of sugar on polarization than on titration with Fehling's solution. At the same time it will be observed that on heating for two hours with hydrochloric acid at 106° F. the polarimetric values become smaller, while the titration values increase.

Dextrin.[2]—In one case of diabetes dextrin appeared to take the place of glucose. It may be recognized by the fact that upon the application of Fehling's test the blue liquid first becomes green, than yellow, and sometimes dark brown. Traces of dextrin are probably present in every urine, but cannot be demonstrated with the common tests.

Laiose.[3]—Laiose has been found in the urine of a diabetic patient. It is essentially characterized by the fact that on titration with Fehling's solution from 1.2 to 1.8 per cent. more sugar is indicated than by the polarimetric method.

Pentoses.—Traces of pentoses probably occur in every urine, but are not demonstrable by the common tests. Somewhat larger amounts may be found after the ingestion of fruit which is rich in pentoses, such as huckleberries, plums, cherries etc. (alimentary pentosuria). The tolerance of pentoses normally is less than 30 to 50 grams. If such amounts are taken one-half usually reappears in the urine.

Marked pentosuria has been described in a morphine habitué by Salkowski and Jastrowitz, where it alternated with glucosuria. Similar cases have been reported by Real, Külz, and Vogel, and others have observed pentosuria in diabetes. Several cases have been described in apparently normal individuals and of late a family tendency to pentosuria has been observed in some cases. In these idiopathic cases arabinose is found, while xylose and rhamnose are met with in the alimentary type of the anomaly.

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[1] Lépine and Boulud, Compt.-rend., vol. cxxxii, p. 610.

[2] Reichard, Maly's Jahresber., 1876, vol. v, p. 60.

[3] Leo, Virchow's Archiv, vol. cvii.

Pentose urines reduce Fehling's solution and Nylander's solution, and give rise to the formation of an osazone when treated with phenylhydrazin. The osazone can be distinguished from that obtained from glucose, maltose, or lactose, etc., by the melting point (159° to 160° C.). The fermentation test is negative. Xylose and rhamnose turn the plane of polarization to the right, while arabinose is optically inactive. The presence of pentoses can be definitely established with the orcin test.

Orcin Test (Bial's Modification[1] of Tollens' Test).—The reagent consists of 1 gram of orcin and 25 drops of the liquor ferri chloridi in 500 c.c. of a 30 per cent. solution of hydrochloric acid. A few c.c. of this are heated to boiling in a test-tube and treated with a few drops of urine. A green color develops in the presence of pentoses. The green pigment can be extracted with amyl alcohol, and on spectroscopic examination it gives rise to a well-defined band of absorption in the red portion of the spectrum near the yellow border.

Tollens' Phloroglucin Test, in which phloroglucin is substituted for the orcin, and in which a deep-red color is obtained in the presence of a pentose, may also be used, but the reagent indicates the presence of glucuronates as well.

LITERATURE.—E. Salkowski u. M. Jastrowitz, "Ueber eine bisher nicht beobachtete Zuckerart im Harn," Centralbl. f. d. med. Wiss., 1892, No. 19. E. Salkowski, "Ueber d. Pentosurie," Berlin. klin. Woch., 1895. No. 17. F. Blumenthal, ibid., No. 26; and Zeit. f. klin. Med., vol. xxxvii, p. 415. E. Külz u. J. Vogel, Zeit. f. Biol., N. F., 1896, vol. xiv, p. 189. E. Salkowski, "Ueber d. Vorkommen von Pentosen im Harn," Zeit. f physiol. Chem., 1899, vol. xxvii, p. 587. Bial, Ueber Pentosurie, Zeit. f. klin. Med., 1900, vol. xxxix, p. 472. Bendix, Münch. med. Woch., 1903, No. 36. Bial, Berlin. klin. Woch., 1904, p. 552.

Glucuronic Acid.

Glucuronic acid is derived from glucose, and constitutes an intermediary product of the normal metabolism of the body. In the urine it is found only in combination with certain fatty and aromatic alcohols, forming compounds which are related to the glucosides and are generally spoken of as the *conjugate glucuronates.* Such bodies have been observed in the urine following the ingestion of chloral, camphor, naphtol, oil of turpentine, menthol, phenol, morphine, antipyrine, etc., and traces may also be obtained from normal urines. The normal glucuronates are undoubtedly compounds of glucuronic acid with phenol, paracresol, indoxyl, and skatoxyl. Their amount is exceedingly small, as the greater portion of these bodies is normally eliminated in combination with sulphuric acid. According to P. Mayer, an increased elimination of glucuronates precedes alimentary glucosuria. Both conditions frequently coexist in diabetic individuals.

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[1] Deutsch. med. Woch., 1903, No. 27.

Of the quantitative variations of the normal glucuronates and their relation to disease, next to nothing is known. Their clinical interest centres in the fact that certain glucuronates are capable of reducing copper and bismuth in alkaline solution. The glucuronates are readily decomposed by boiling with 1 per cent. H_2SO_4 (for one to five minutes). Unless this is previously done reduction of the alkaline copper sulphate solution only takes place slowly on prolonged heating. But if the cleavage is first accomplished it occurs at once. Such urines do not undergo fermentation. The glucuronates turn the plane of polarization to the left, while glucuronic acid itself is dextrorotatory. Like the pentoses, the glucuronates give a positive reaction with phloroglucin, while they do not react with orcin (see above). With the free acid phenylhydrazin forms crystalline compounds.

A quantitative method has recently been published by Neuberg and Neumann.[1]

LITERATURE.—H. Thierfelder, "Ueber d. Bildung v. Glykuronsäure," etc. Zeit. f. physiol. Chem., 1886, vol. x, p. 163; "Untersuchungen über d. Glykuronsäure," ibid., 1887, vol. xi, p. 388. P. Mayer, "Ueber d. Ausscheidung u. [illegible] Nachweis d. Glykuronsäure," Berlin. klin. Woch., 1899, pp. 591 and 617. [illegible] Mayer u. C. Neuberg, Zeit. f physiol. Chem., 1900, vol. xxix, p. 256

Inosit.

According to Hoppe-Seyler, traces of inosit may be found in the urine under normal conditions. Somewhat larger quantities are eliminated following the ingestion of large amounts of water, and for this reason possibly inosituria is notably observed in cases of diabetes insipidus, in diabetes mellitus, and in chronic interstitial nephritis. Its occurrence in these diseases is, however, not constant. The substance is devoid of clinical interest. It is not a carbohydrate, but belongs to the aromatic series, and is commonly regarded as hexahydroxybenzol. Its formula is $C_6H_{12}O_6+H_2O$. For methods of isolating the substance from the urine, the reader is referred to special works.[2]

Urinary Pigments and Chromogens.

Under normal conditions urochrome and uroerythrin, to which latter the red color of urate sediments is due, are the only pigments which occur preformed in the urine. In disease, on the other hand,

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[1] Zeit. f. physiol. Chem., 1905, vol. xliv, p. 127.

[2] C. E. Simon, Physiological Chemistry, Lea Bros. & Co.

ous other pigments may be found, which occur either free or
he form of chromogens. Among the former may be mentioned
oglobin, methemoglobin, hematin, hematoporphyrin, urorubro-
atin, urofuscohematin, urobilin, the biliary pigments, and melanin;
e abnormal chromogens are met with following the ingestion of
in drugs, such as santonin, senna, rheum, iodine, etc., as also in
of poisoning with carbolic acid, creosote, etc. The occurrence
me of these substances, such as the various forms of blood pig-
, the biliary pigments, and indigo, viz., indican, is of considerable
al interest, while others again are of only minor importance.

rmal Pigments. Urochrome.—To the presence of this pig-
, which appears to be identical with the *normal urobilin of*
Munn, but which should not be confounded with the *pathological*
lin of Jaffé, the normal yellow color of the urine is probably
ly due. It is supposedly derived from bilirubin, which in turn is
able to hematin, and thus from the hemoglobin of the blood.

order to obtain urochrome from normal urine, this is acidulated
1 to 2 grams of dilute sulphuric acid pro liter, filtered, and
rated with ammonium sulphate in substance, when the flakes
h are formed, if an excess of the salt has been added, are dried
treated with warm, slightly ammoniacal absolute alcohol; the
ent is then obtained upon evaporation of the alcohol.

n alcoholic solution of urochrome, like the urobilin of Jaffé,
bits a beautiful greenish fluorescence when treated with ammonia
a few drops of a solution of zinc chloride; but, unlike the latter
tance, its acidulated alcoholic solutions present a broad band of
rption at *F*, which extends more to the left than to the right of
line, while the remainder of the spectrum is at the same time
rbed to the right end, from a point somewhat to the left of *G*.
rod, on the other hand, states that by acting upon urochrome with
s he did not succeed in obtaining any product showing the urobilin
l or yielding the well-known fluorescence with zinc chloride and
onia. But a substance having both these properties was readily ob-
ed by the action of aldehyde upon an alcoholic solution of the pig-
t. In a short time—shorter still when the liquid is warmed—an
rption band appears like that of urobilin, and the tint of the solu-
deepens to a rich orange-yellow. With zinc chloride and ammonia
illiant green fluorescence appears, and the band is shifted toward
ed, as that of urobilin is under like circumstances. The process
be stopped at this point by the simple addition of water, for
hyde has no such action upon aqueous solutions of urochrome.
owever, the action be allowed to continue, a further change
es; the liquid reddens, and a second band appears in the violet.
fluorescence can still be obtained with zinc chloride and am-
ia, and both bands are shifted toward the red and are closer
ther than before. The reaction with aldehyde, according to

Garrod, affords a very delicate test for the presence of urochrome in alcoholic solutions. The product of the earlier stage, although it is not identical with urobilin, resembles that pigment quite as closely as the products obtained from bilirubin and hematin by the action of reducing agents; but no second band is developed when aldehyde is added to an alcoholic solution of urobilin.[1]

By the action of potassium permanganate upon urobilin Riva and Chiodera[2] obtained a substance closely resembling urochrome, and a similar product is formed when an aqueous solution of urobilin containing ether is evaporated upon a water bath. Neither product shows any absorption band, and both behave as urochrome does when it is acted upon by aldehyde.

Uroerythrin.—Uroerythrin is the pigment which imparts the red color to crystals of uric acid and the pink tint to urate sediments. Under strictly normal conditions it probably does not occur in the urine, but it readily appears with the slightest deviation from health, and when present in larger amounts imparts a deep-orange color to the urine. Under pathological conditions it is seen especially in cases of hepatic insufficiency, in which the liver, owing to a greatly increased destruction of red corpuscles, is unable to transform into bile pigment all the blood pigment which is carried to it. It also occurs when an absolute insufficiency on the part of the hepatic cells exists, so that the organ is not even capable of causing the transformation of a *normal* amount of hemoglobin. Uroerythrin is seen in notable quantities in cases of cirrhosis and carcinoma of the liver, in passive congestion resulting from heart disease, in acute articular rheumatism, gout, pneumonia, malarial fever, erysipelas, spinal curvature, etc. In typhoid fever a marked excretion of uroerythrin is exceptional, and its occurrence has been associated with pulmonary complications. In nephritis it is seldom found in the urine, but Garrod cites an instance of pneumonia in which an abundant excretion of the substance accompanied conspicuous albuminuria.

In certain diseases, such as hepatic cirrhosis, the excretion of uroerythrin, as also of urobilin, is said to be much diminished when the patient is placed upon a milk diet (Riva).

When present in large amounts uroerythrin is readily recognized by the salmon-red color which it imparts to urinary sediments. Otherwise it is best to precipitate the urine with neutral lead acetate, barium chloride, or a similar reagent, when in the absence of uroerythrin a milky-white precipitate is obtained, while a pale rose-colored sediment indicates the presence of the pigment in appreciable amounts; a more pronounced rose color is produced if large quantities are present. In every case at least ten to fifteen minutes should allowed to elapse before forming a definite conclusion, so that the liment may have abundant time to settle.

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[1] A. E. Garrod, "The Bradshaw Lecture on the Urinary Pigments in their Pathological Aspects," Lancet, Nov. 10, 1900.

[2] Arch. ital. di Clin. Med., 1896, vol. xxxv, p. 505.

Normal Chromogens.—The chromogens occurring in normal urine indican, urohematin, and an unknown chromogen which yields rosein when treated with mineral acids.

Indican.—It has been pointed out (see Sulphates) that the indol med during intestinal putrefaction is oxidized to indoxyl in the od; this, entering into combinaton with sulphuric acid, is eliminated in the urine as sodium or potassium indoxyl sulphate, or indican. Formerly it was thought that indican was also formed within tissues of the body in the absence of putrefactive organisms (Salwski).[1] Further researches, however, have demonstrated that microganisms are always concerned in the production of indican, and that health the large intestine is its sole source. Baumann, who ceeded in disinfecting the intestinal tract of a dog by means of ge doses of calomel, observed that all traces of indican, as also of enol and paracresol, disappeared from the urine. According to nator, moreover, indican does not occur in the urine of newly born ants which have not as yet received nourishment. Tuczek's servations on abstinence from food in cases of insanity, in which lican was observed in the urine only when albumins, though in nimal amounts, were ingested, also speak very strongly against lkowski's theory. Finally, it has been demonstrated that in cases which an artificial anus is established near the distal end of the um the conjugate sulphates disappear almost entirely from the ne, while they reappear in normal amount as soon as the connection tween the small and large intestines has been reëstablished.[2]

The amount of indican which is normally eliminated in the urine ries somewhat with the character of the diet. Jaffé[3] obtained 6.6 rms. from 1000 c.c. of urine, as an average of eight observations. le largest quantities excreted in health are found after a liberal lulgence in animal food, while the smallest amounts are observed ring a milk or kefir diet. By means of the latter article, indeed, greatest diminution in the degree of intestinal putrefaction may effected in man.

In pathological conditions an increased elimination of indican is served:

I. In all diseases which are associated with an increased degree intestinal putrefaction. As there appears to be little doubt that s is largely regulated by the acidity of the gastric juice, an in-

Ber. d. deutsch. chem. Ges., 1876, vol. ix, pp. 138 and 408. Baumann, Zeit. hysiol. Chem., 1886, vol. x, p. 123. Senator, Centralbl. f. d. med. Wiss., 7, vol. xv, pp. 357, 370, and 388.

Nencki, Macfadyen u. Sieber, Arch. f. exper. Path u. Pharmakol., 1891, vol. x.

Centralbl. f. d. med. Wiss., 1872, vol. x, pp. 2, 481, and 497; and Virchow's hiv, 1877, vol. lxx, p. 72.

creased indicanuria, according to personal observations, is encountered when anachlorhydria or hypochlorhydria exists. Large quantities of indican are thus eliminated in cases of carcinoma of the stomach, and exceeded only by those observed in cases of ileus; so that this symptom, in my estimation, is of considerable value in differential diagnosis, and is one, moreover, which has not received the attention it deserves. Exceptions to this rule are at times, though rarely, met with, for which it is, however, impossible to account at present. Large quantities of indican are also observed in cases of acute, subacute, and chronic gastritis. In the course of personal observations in this direction I was impressed with the curious phenomenon that in cases of ulcer of the stomach, notwithstanding the simultaneous occurrence of hyperchlorhydria, an increased elimination of indican, contrary to what is usually seen in hyperchlorhydria referable to other causes, is quite commonly found. Possibly the existence of muscular atony which was noted in these cases may serve to explain this apparent incongruity, but it is as yet impossible to offer a satisfactory explanation of the phenomenon. Remembering the origin of indican, and the relation which the amount eliminated bears to the degree of intestinal putrefaction, it will be unnecessary to enumerate the long list of diseases in which an increased indicanuria has been observed, as it will be found that in the majority of these cases the indicanuria is merely an index of the condition of the gastric juice and the motor power of the stomach.[1]

2. It should be noted that in cases in which the peristaltic movements of the *small* intestine have become impeded, as in ileus, acute and chronic peritonitis, an increased elimination of indican will invariably take place, no matter what the state of the gastric juice may be. In such conditions, and especially in ileus, the largest quantities are observed, a point which may be of *decided* value in differential diagnosis, as diseases of the large intestine alone are *never* associated with an increase in the amount of indican. *In simple, uncomplicated constipation increased indicanuria is not seen;* and should an examination in such cases reveal the presence of more indican than normal, it will be safe to assume the existence of disease elsewhere, and especially of the stomach.

3. As albuminous putrefaction may also take place within the body, an increased indicanuria is observed in cases of empyema, putrid bronchitis, gangrene of the lung, etc.; but while in the conditions mentioned above the indol-producing organisms appear to be especially active, the elimination of phenol in the latter condition may be more pronounced at times than that of indican. Bearing in mind the points here set forth, I cannot agree with others in saying

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[1] C. E. Simon, "Indicanuria," Am. Jour. Med. Sci. (full literature), 1895, vol cx, p. 48.

that the study of indicanuria possesses no importance from a clinical standpoint. I maintain, on the other hand, that *an examination of the urine in this direction is at least as important as the testing for albumin and sugar, and that points of decided importance, not only in diagnosis, but also in treatment, may thus be gained.*

Of interest in this connection is the observation that in cases of increased indicanuria oxalate sediments are not uncommonly observed; but I am not willing to admit, as Harnack and van der Leyen suggest, that the indicanuria which follows the ingestion of small doses of oxalic acid is produced by a toxic action of the acid upon the tissue albumins. In these cases also the increased indicanuria is referable to increased intestinal putrefaction.[1]

When indican is treated with hydrochloric acid, it is decomposed into sulphuric acid and indoxyl; should an oxidizing substance be present at the same time, indigo blue, the blue coloring matter of the urine, results:

$$\underset{\substack{\text{Potassium indoxyl}\\ \text{sulphate.}}}{2C_8H_6NKSO_4} + 20 = \underset{\text{Indigo blue.}}{C_{16}H_{10}N_2O_2} + 2HKSO_4.$$

Indigo blue in small amounts may be found free in the sediment of decomposing urines, usually occurring in the form of small, amorphous granules, more rarely in crystalline form. Urines have, however, also been observed which were blue when passed, or which turned blue as a whole upon standing. Such a phenomenon must be regarded as a medical curiosity. Undoubtedly it is referable to the action of microörganisms (see Bacteriuria), although McPhedran and Goldie mention that in their case bacteria were present only in small numbers.[2]

The blue pigment which may be obtained from urines has been variously described as Prussian blue, urocyanin, cyanurin, Harnblau, uroglaucin, choleraic urocyanin, but it has been shown to be indigo blue, and derived from its colorless antecedent indican. This has been shown to be identical with the uroxanthin of Heller and Thudichum's choleraic urocyaninogen.

TESTS FOR INDICAN.—A few cubic centimeters of urine are mixed with an equal volume of Obermayer's reagent, and shaken with a small amount of chloroform, which last takes up the indigo blue which is formed. The resultant extract is normally either colorless or of a light sky blue; a darker color indicates an increased amount of indican. *Obermayer's reagent* is a 2 pro mille solution of ferric chloride in concentrated hydrochloric acid.[3]

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[1] v. **Moraczewski**, "Oxalurie and Indicanurie," Cent. f. inn. Med., 1903, No. 1.

[2] A. **McPhedran** and W. Goldie, "A Case of Indigosuria," Trans. Assoc. Am. Phys., 1901, vol. xvi, p. 242.

[3] Wien. klin. Woch., 1890, vol. iii, p. 176.

Stokvis' modification of Jaffé's test may also be employed.[1] To this end a few cubic centimeters of urine are treated with an equal volume of concentrated hydrochloric acid, and 2 or 3 drops of a strong solution of sodium or calcium hypochlorite. The mixture is shaken with 1 or 2 c.c. of chloroform as above. The indigo which is set free in this manner is taken up by the chloroform, and colors this blue to a greater or less extent, the degree of increase, as compared with the normal, being determined by the intensity of the color. Albumin need not be removed. Bile pigment, which interferes with the reaction, is removed by means of a solution of lead subacetate. Urines presenting a very dark color may be cleared in the same manner. Potassium iodide, owing to the liberation of free iodine, will color the chloroform a rose red.

For the sake of comparison, it is well to employ the same quantities of urine and reagents in every case, marked tubes being very convenient for this purpose.

QUANTITATIVE ESTIMATION. *Wang's Method.*[2]—The method is based upon the decomposition of potassium indoxyl sulphate by means of concentrated hydrochloric acid and the oxidation to indigo-blue of the indoxyl which is thus formed. The indigo blue is further transformed into indigo-sulphuric acid, and this titrated with a solution of potassium permanganate of known strength.

Reagents required: 1. A 20 per cent. solution of lead acetate.

2. Obermayer's reagent. This is a 2 pro mille solution of ferric chloride in concentrated hydrochloric acid (sp. gr. 1.19).

3. Chloroform.

4. Concentrated sulphuric acid.

5. A mixture of equal parts of alcohol (96 per cent.), ether, and water.

6. A solution of potassium permanganate containing about 3 grams pro liter The titration is conducted with this solution diluted in the proportion of 5 c.c. to 195 c.c. of water. Its titre is ascertained before each titration by comparing it with a dilute solution of oxalic acid of known strength; for example, one containing 0.1 gram of the acid dissolved in 100 c.c. of water, as described on page 426. The amount of indigo blue which each cubic centimeter will represent is ascertained by multiplying the corresponding amount of oxalic acid by 1.04.

Example.—Supposing that the permanganate solution is found of such strength that 1 c.c. represents 0.00014 gram of oxalic acid; the corresponding amount of indigo would be 0.00014 × 1.04= 0.00015 gram.

Method.—The urine is first examined for indican, as described above. Should a very intense reaction be thus obtained, only 25 or

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[1] See Senator, Centralbl. f. d. med. Wiss., 1877, vol. xv, p. 257.

[2] "Ueber d. quantitative Bestimmung d. Harnindikans," Zeit. f. physiol. Chem., vol. xxv, p. 406.

50 c.c. are used for the quantitative estimation, while larger amounts are taken (200 to 500 c.c.) if the reaction is of only moderate intensity or negative altogether.

The urine is precipitated with lead acetate solution, care being taken to avoid an excess. A large and accurately measured portion of the clear filtrate is treated in a separating funnel with an equal volume of Obermayer's reagent and extracted with chloroform. To this end 30 c.c. are added at a time and shaken for one minute. Two or three extractions are usually sufficient to remove the entire amount of indigo. The extract is placed in a small flask, and the chloroform distilled off. The residue is dried for a few minutes on a water bath until traces of remaining chloroform have been removed. It is then washed with the alcohol-ether-water mixture to remove the reddish-brown pigment which is present together with the indigo blue. The latter remains undissolved. After filtering off any particles of indigo that may be in suspension, through a small filter, this is dried and repeatedly extracted with boiling chloroform. The chloroform extract is filtered into the original indigo flask, the chloroform distilled off, the residue dried as before, and while still warm treated with 3 or 4 c.c. of concentrated sulphuric acid. The entire residue should be brought into solution by careful agitation. After standing or twenty-four hours the contents of the flask are poured into 100 c.c. of cold water; the flask is rinsed and the washings added to the solution. This is filtered once more and titrated with the permanganate solution. At first the blue color of the solution changes but little; later it turns greenish, and finally becomes yellowish or entirely colorless—not red. As a rule, the end reaction is quite distinct, but the titration requires experience. The best results are obtained if from 10 to 15 c.c. of the dilute permanganate solution are used. The resulting amount of indigo contained in the measured-off quantity of the first filtrate is then ascertained as described above.

Example.—Amount of urine: 1780 c.c.

The stock solution of potassium permanganate contains 3 grams to the liter; 1 c.c.=0.00596 gram of oxalic acid=0.0062 gram of indigo. Diluted solution (5 to 200); 1 c.c.=0.00015 gram of indigo. 300 c.c. of urine were precipitated with 25 c.c. of the lead solution; 250 c.c. of the filtrate, corresponding to 230.7 c.c. of urine, treated with 250 c.c. of Obermayer's reagent. Extracted twice with chloroform. 4.3 c.c. of the permanganate solution were used in the titration=0.00065 gram of indigo, correspondng to 0.005 gram in the 1780 c.c., according to the equation:

$$230.7 : 0.00065 :: 1780 : x; \; x = \frac{1.157}{230.7} = 0.005.$$

Other methods for the quantitative estimation of indican which have heretofore been used, with the exception of the spectroscopic method of Müller, are not only inaccurate, but, like this, too time consuming and complicated to be of value to the practising physician. As a consequence almost all observers have based their conclusions upon an approximative estimation only. For practical purposes this is sufficient, and even Wang's method, though accurate and simple, will hardly find a ready entrance into the clinical laboratory, as it is still too time consuming and too expensive for daily use.

Other quantitative methods are those of Ellinger[1] and Strauss,[2] which should be read in the original.

Urohematin.[3]—Urohematin appears to be the chromogen of the red pigment of the urine, and is very likely closely related to indoxyl. Little is known of its chemical composition or of its mode of formation. In all probability the red pigment which may be obtained from this substance is identical with other red pigments which have been described from time to time as occurring in the urine, such as that of Scherer, the urrhodin of Heller, the urorubin of Plosz, Schunk's ind rubin, Bayer's indigo purpurin, Giacosa's pigment, and also the indigo red obtained by Rosenbach and Rosin by oxidation of the urine with nitric acid.

Further investigations are necessary before this subject is fully understood; but bearing in mind the probable origin of urohematin from indoxyl, it would possibly be best to speak of the red pigment as indigo red. In accordance with the view that urohematin is an indoxyl derivative, its clinical significance is similar to that of indican (which see).

Test.—The presence in normal urine of urohematin—*i. e.*, a chromogen yielding a red pigment when treated with certain reagents—may be demonstrated by shaking urine with chloroform and decanting after several days, when the addition of a drop of hydrochloric acid to the chloroform extract will cause the appearance of a beautiful rose color; this varies in intensity according to the amount of the chromogen present.

The purplish color so often obtained in the chloroform extract when Stokvis' modification of Jaffé's indican test is employed is due to a mixture of indigo blue and indigo red. Indican, however, is generally present in larger amounts than urohematin. In normal and, usually also, in pathological urines a red color is not obtained with the test mentioned. In a few isolated cases of ileus, peritonitis, and carcinoma of the stomach I have found more indigo red than indigo blue.

The so-called "Reaction of Rosenbach" is a convenient test for indigo red when this is present in increased amounts; the boiling urine is treated drop by drop with concentrated nitric acid, when in the presence of large amounts of indigo red it assumes a dark Burgundy color, which sometimes takes on a bluish tinge when held to the light. Owing to a precipitation of the pigment the mixture at the same time becomes cloudy and the foam assumes a blue color. In well-marked cases the Burgundy color does not appear to be changed by the further addition of nitric acid, but will sometimes suddenly change from red to yellow when 10 to 20 drops of the acid have been added.

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[1] Zeit. f. phys. Chem., vol. xxxviii, p. 178.

[2] Deutsch. med. Woch., 1902, April, 17.

[3] G. Harley, Verhandl. d. physik. med. Ges. z. Würzburg, 1855, vol. v, p. 1.

This reaction Rosenbach[1] regarded as symptomatic of various forms of severe intestinal disease associated with an impeded resorption throughout the entire intestinal tract. Ewald[2] likewise noted this reaction in cases of extensive disease of the small intestine, in carcinoma of the stomach, and in acute and chronic peritonitis; but he obtained negative results in carcinoma of the colon, stricture of the esophagus, chronic diarrhea, etc. *Rosenbach's reaction should be viewed in the same light as a highly incresed elimination of indican.* I have met with the reaction in all conditions associated with greatly increased intestinal putrefaction, and, like Ewald, failed to note the reaction in a few cases of occulsion of the large intestine, in which an increased elimination of indican is likewise never observed.

Uroroseinogen.[3]—In addition to indican and urohematin, still another chromogen, which yields a rose-red pigment when treated with mineral acids, appears to occur in normal urine, although in small amounts. It is commonly regarded as a skatol derivative. The pigment, which has received the name *urorosein,* or Harnrosa, appears to be identical with Heller's urophain. Urorosein is best demonstrated by treating 5 to 10 c.c. of urine with an equal amount of concentrated hydrochloric acid, and 1 or 2 drops of a concentrated solution of sodium hypochlorite, when in the presence of much indican the mixture assumes a dark-greenish, blackish, or dark-blue color, owing to the formation of indigo. When the mixture is shaken with chloroform the supernatant fluid exhibits a beautiful rose color, which is due to the urorosein. This may now be extracted with amyl alcohol and separated from other pigments which are present at the same time, by shaking with sodium hydrate, whereby the solution is decolorized. Upon the addition of a drop or two of hydrochloric acid to the alcoholic extract the rose color reappears. Such solutions, however, soon become decolorized upon standing. A rose-red ring, referable to this pigment, is also frequently obtained in pathological urines when the ordinary nitric acid test is employed.

While normally urorosein is obtained only in traces, appreciable amounts are often met with in pathological conditions associated with grave disturbances of nutrition, as in nephritis, diabetes, carcinoma, dilatation of the stomach, pernicious anemia, typhoid fever, phthisis, and at times in profound chlorosis, etc. A vegetable diet also appears to cause an increase in the amount of the chromogen.

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[1] Berlin. klin. Woch., 1889, vol. xxvi, pp. 5, 490, and 520, and 1890, vol. xxvii, p. 585.

[2] Ibid., 1889, vol. xxvi, p. 953.

[3] H. Rosin, Deutsch. med. Woch., 1893, p. 51.

Pathological Pigments and Chromogens. The Blood Pigments. —The blood pigments proper which may occur in the urine have already been considered and in this connection it will only be necessary to refer briefly to the occasional presence of hematin, urorubrohematin, and hematoporphyrin.

HEMATIN is only rarely found. In order to demonstrate its presence, the urine is rendered strongly alkaline with ammonia, filtered, and the filtrate examined spectroscopically. (See Blood.)

URORUBROHEMATIN and UROFUSCOHEMATIN have been observed only once by Baumstark[1] in the urine of a case of pemphigus leprosus complicated with visceral lepra; they appear to be closely related to hematin.

HEMATOPORPHYRIN.—McMunn found a pigment answering the description of this substance in the urine in cases of rheumatism, Addison's disease, pericarditis, and paroxysmal hemoglobinuria, which he termed urohematin, but which in all probability was hematoporphyrin. Le Nobel found the same pigment in two cases of hepatic cirrhosis and in one case of croupous pneumonia. Others have likewise met with hematoporphyrinuria in various forms of hepatic disease, as also in phthisis, exophthalmic goitre, typhoid fever, and hydroa æstivalis; further, in association with intestinal hemorrhages, in cases of lead poisoning, and especially during long-continued use of sulphonal, trional, and tetronal. Nebelthau records the history of a female patient, the subject of congenital syphilis, who had passed dark-red urine as long as she could remember, and continued to do so while under observation. Stern mentions a case in which marked hematoporphyrinuria was associated with icterus in a glucosuric individual. Recent researches, moreover, have shown that in traces at least the substance is present in every urine. As regards the origin of these normal traces, the evidence is in favor of the view that they are formed within the body during its normal metabolism, and most likely in the liver, whence the substance is eliminated in the bile. A portion then escapes with the feces, while a similarly small amount is resorbed and eliminated in the urine. Increased amounts would accordingly suggest the existence of a hepatic insufficiency; and, as a matter of fact, we find that actual anatomical lesions then not infrequently occur. Taylor and Sailer thus report that in their case of sulphonal poisoning widespread degeneration of the hepatic cells existed; and Neubauer was able to isolate the pigment from the er of rabbits to which sulphonal had been administered, while it as absent in all other organs. On the other hand, it is difficult to cribe all the phenomena of such hematoporphyrinuria to hepatic anges, seeing that changes of like degree may occur without conicuous urinary abnormality, and there is still much that is obscure this condition.

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[1] Pflüger's Archiv, 1874, vol. ix, p. 568. See, also, J. W. Schultz, Diss., Greifswald, 1874.

Stokvis attributed the increased elimination of hematoporphyrin cases of lead poisoning and following the continued use of sulhonal to the occurrence of hemorrhages into the intestinal mucosa, nd suggested that the transformation of the hemoglobin into ematoporphyrin was favored by the sulphonal. But while intestial hemorrhages may occur in the sulphonal cases, they are not lways observed, and, as Garrod points out, Kast and Weiss, as lso Neubauer, were unable to verify the recorded experiments of tokvis, in which he claims to have obtained a small amount of ematoporphyrin when fresh blood was digested with pepsin-hydrohloric acid and sulphonal at from 38° to 40° C.

Urines which contain much hematoporphyrin are usually dark ed in color, but the shade may vary from a sherry or port-wine int to a dark Bordeaux. It is noteworthy, however, that this color s not primarily due to the exaggerated degree of hematoporphyinuria, but, as Hammarsten first pointed out, to other abnormal igments which are but little known, but which are probably closely elated to hematoporphyrin. As Garrod says, the removal of he hematoporphyrin from such urines causes little or no change f color, and when this pigment is added to normal urine until on pectroscopic examination bands of similar intensity are seen, the hange of tint produced is comparatively slight. In one such case, ot due to sulphonal, he was able to isolate a purple pigment which iffered in its properties from any known urinary coloring matter, nd to which the color of the urine in question was obviously in the ain due. Neumeister also states that in sulphonal intoxication an ron-containing derivative of hemoglobin occurs in the urine, which resents a reddish-violet color and shows a single band of absorption the blue portion of the spectrum immediately bordering on the green.

Albumin is not present in uncomplicated cases of hematoporhyrinuria, and the pigment itself does not give the albumin reactions.

To demonstrate the presence of hematoporphyrin under normal onditions, or when small amounts only are present in the urine, arrod's method should be employed.

Garrod's Method.—Several hundred c.c. of urine (500 to 1500) are reated with a 10 per cent. solution of sodium hydrate in the proporion of 20 c.c. of the alkali solution for 100 c.c. of urine. The preipitated phosphates are filtered off and thoroughly washed by epeatedly suspending them in water. Should the precipitate be of reddish color, or if it shows the spectrum of hematoporphyrin in alkaline solution when examined on the filter in the moist state, we may conclude that much hematoporphyrin is present. In this case it is washed until the filtrate is colorless. If traces only are present, however, one washing must suffice. The precipitate is then treated with alcohol, which is acidified with hydrochloric acid to such an extent that the phospates are entirely dissolved. The resulting solution should not exceed 15 to 20 c.c. in volume. This is then examined in a layer, of not less than 3 to 4 cm. in thickness, for the spectrum of acid hematoporphyrin, using a spectroscope with slight dispersion. The solution is now rendered alkaline with ammonia and treated with an amount of acetic acid which just suffices to redissolve the precipitated phosphates. On shaking with chloroform this extracts the pigment, and the chloroform solution then gives the spectrum of the alkaline hematoporphyrin, since organic acids do not change the pigment to the form which yields the acid spectrum. The residue which remains after evaporating the chloroform can finally be washed with water and dissolved in alcohol, when a nearly pure solution is obtained, which is comparable with a solution of hematoporphyrin obtained from hematin.

Precautions: If a preliminary test shows that the urine contains but little phosphates, a small quantity of calcium phosphate in acetic acid is added before the urine is rendered alkaline with the sodium hydrate solution. As hematin and chrysophanic acid are also precipitated with the phosphates, their absence must be ensured. For this reason the urine should contain no rhubarb or senna.

In conclusion, it may be said that a chromogen of hematoporphyrin is also usually present in urines containing the free pigment, which probably explains why such urines gradually become darker on standing.

LITERATURE.—A complete account of the literature on hematoporphyrinuria up to 1893 is given by R. Zoja, "Su gualche pigmento di alcune urine," etc., Arch. ital. di clin. med., 1893, vol. xxxii, p. 63. A. E. Garrod, loc. cit.; and Centralbl. f. inn. Med., 1897, No. 21. Taylor and Sailer, Contributions from the William Pepper Laboratory, Philadelphia, 1900, p. 120. O. Neubauer, Arch. f. exper. Path. u. Pharmakol., 1900, vol. xliii, p. 455. B. J. Stokvis, "Zur Pathogenese d. Hæmatoporphyrinurie," Zeit, f. klin. Med., vol. xxviii, p. 1. Kast u. Weiss, Berlin. klin. Woch., 1896, vol. xxxiii, p. 621. Hammarsten, "Skandin. Arch. f. Physiol.," 1891, vol. iii, p. 31. Neumeister, Physiol. Chem., Jena, 1897. Nebelthau, Zeit. f. physiol. Chem., 1899, vol. xxvii, p. 324. B. Ogden, Boston Med. and Surg. Jour., 1898

Biliary Pigments.—Of the four biliary pigments, viz., bilirubin, biliverdin, biliprasin, and bilifuscin, the former alone is met with in freshly voided urines, while the others may form upon standing, being oxidation products of bilirubin. The pigment is never found in normal urine, and its occurrence may be regarded as a positive symptom of disease.

In health it will be remembered that BILIRUBIN is formed in the liver from blood pigment, and is eliminated into the small intestine, which it is transformed into hydrobilirubin and largely excreted as ch in the feces, while a small portion is reabsorbed into the blood nd eliminated in the urine as urochrome or normal urobilin. When-er, then, the outflow of bile into the intestines becomes impeded lirubin is absorbed by the lymphatics and eliminated in the urine.

Among the numerous causes which give rise to *choluria* under ach conditions may be mentioned obstruction of the biliary ducts, nd especially of the common duct, referable to simple swelling of ts mucous membrane, as in the ordinary forms of catarrhal jaundice. It may also be due to the presence of a biliary calculus, to parasites, compression of the duct by tumors of the liver, the gall bladder, the duct itself, and of neighboring structures, and particu-arly of the pancreas, stomach, and omentum. Whenever the blood pressure in the liver is lowered, so that the tension in the smaller biliary ducts becomes greater than that in the veins, choluria ikewise results. The icterus occurring under all such conditions has been termed *hepatogenic icterus*, in contradistinction to the form observed in cases in which the liver has either totally or partially ost the power of forming bile, be this owing to the existence of degenerative processes affecting its glandular epithelium, as in cases of acute yellow atrophy, or to destruction of red corpuscles going on so rapidly and so extensively that the organ is incapable of transforming into bilirubin all the blood pigment which is carried to t. This occurs in some cases of pernicious anemia, malarial intoxi-cation, typhoid fever, poisoning with arsenious hydride, etc. Icterus neonatorum is probably to a certain extent also dependent upon the atter cause. To this form the term *hematogenic icterus* has been applied. In such cases the occurrence of bilirubin in the urine can only be explained by assuming that a transformation of blood-coloring matter into bilirubin has taken place in the blood itself or in other issues of the body. As a matter of fact, it appears to be generally accepted that such a transformation *can* occur outside of the liver, as the hematoidin which may be found in old extravasations of blood seems to be identical with bilirubin. On the other hand, however, the existence of a hematogenic icterus is positively denied, especially by Stadelmann. In accordance with his view it may be demonstrated that in cases of pernicious anemia, malaria, etc., the urine does not contain bilirubin, but usually urobilin. In cases of this kind which I had occasion to examine, bilirubin was, as a matter of fact, never found. Further investigations are necessary to settle this question.

Usually the presence of biliary pigment may be recognized by direct inspection, as urines which contain it in notable amounts present a color varying from a bright yellow to a greenish brown. Any morphological elements which may occur in the sediment are stained a golden yellow, and the same color is imparted to the foam of the urine as well as to the filter paper used in the filtration. At times, however, and particularly in cases in which the icterus is only beginning to appear, the presence of bilirubin is not infrequently overlooked, and urines containing urobilin in large amounts may be similarly mistaken for icteric urines. In doubtful cases, therefore, whether icterus exists or not, but in which the urine presents an intense yellow color, it is necessary to have recourse to chemical tests. A large number of these have been devised, all of which are fairly reliable. Only those will be described which I have examined myself and which are especially delicate.

Smith's Test.[1]—5 to 10 c.c. of urine are placed in a test-tube and treated with 2 or 3 c.c. of tincture of iodine (which has been diluted with alcohol in the proportion of 1 to 10) in such a manner that the iodine solution forms a layer above the urine. In the presence of bilirubin a distinct emerald-green ring is seen at the zone of contact. This test can be highly recommended, as it is exceedingly simple and not surpassed in delicacy by any other.

Huppert's Test.[2]—10 to 20 c.c. of urine are precipitated with milk of lime (a solution of barium chloride is, perhaps, still more convenient), and the precipitate after filtering brought into a beaker by perforating the filter and washing its contents into the latter with a small amount of alcohol acidualted with sulphuric acid. The mixture is boiled, when in the presence of bilirubin the solution assumes a bright emerald-green color. Huppert's test is as delicate as is that of Smith, but is not so convenient for the needs of the practising physician.

Gmelin's Test (as modified by Rosenbach).[3]—The urine is filtered through thick Swedish filter paper, when the latter is removed and a drop of concentrated nitric acid, which has been allowed to stand exposed to the air for a short time, is placed upon its inner surface. In the presence of bilirubin a prismatic play of colors will be seen to occur around the nitric acid spot.

Gmelin's Test.[4]—The urine is treated with nitric acid, which is carried to the bottom of the test-tube by means of a pipette, so as to form a layer beneath the urine, when a color play, as already described (p. 463), will take place at the line of contact between the two fluids; the green color is the most characteristic.

In this connection a few words may also be said of the occurrence in the urine of biliary acids and cholesterin.

Biliary Acids.—These may usually be found in the urine whenever bile pigment is present, so that their clinical significance is essen-

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[1] Dublin Med. Jour., 1876, p. 449.

[2] Arch. d. Heilk., 1867, vol. viii, pp. 351 and 476.

[3] Centralbl. f. d. med. Wiss., 1876, vol. xiv, p. 5.

[4] Tiedemann u. Gmelin, Die Verdauung nach Versuchen, Heidelberg, 1826, i, p. 80.

the same as that attaching to bilirubin. Their demonstration is,
ver, attended with much difficulty (see Feces).

olesterin.—Cholesterin has never been found in icteric urines,
is only rarely seen in other pathological conditions. It has
observed in cases of chyluria, fatty degeneration of the kidneys,
etes, in one case of epilepsy, in eclampsia, and in several cases
egnancy. v. Jaksch noted cholesterin crystals in a urinary sedi-
in a case of tabes and cystitis. Glinsky records a similar obser-
n. Harley found it repeatedly in cases of pyuria. Reich states
he found cholesterin crystals of the size of a dollar in the urine of
se of chronic cystitis. Hirschlaff found larger quantities in the
of a case of hydronephrosis; on one occasion 5.8 grams in 100
of urine. I have found cholesterin crystals in the sediment in
se of acute nephritis. Güterbock described a urinary calculus
ined from the bladder of a woman which consisted almost
ely of cholesterin (see also Feces). Langgaard noted the pres-
of the substance in a case of chyluria.[1]

thological Urobilin.—This pigment should not be confounded
the urochrome or normal urobilin described above, to which
closely related, but from which it may be distinguished by means
e spectroscope. Gautier states that pathological urobilin may
btained from urochrome by submitting the latter to the action
ducing agents; and, as I have already pointed out, Riva and
dera obtained a substance from urobilin by the action of potas-
permanganate, which closely resembles urochrome. It is said
e identical with the *stercobilin* found in the feces, but differs
Maly's hydrobilirubin in containing a much smaller percentage
itrogen, viz., 4.11, as compared with 9.22 (Garrod and Hop-
. While its occurrence in the urine is essentially a pathological
omenon, it is at times also met with in normal urine, and
ars to be derived from a special chromogen, *urobilinogen,* from
h it may be set free by the addition of an acid. Both urobilin
its chromogen are precipitated by saturating the urine with
onium sulphate, and both are soluble in chloroform. Accord-
o Maly, urobilin is formed by the reduction of bilirubin in the
tine, and is then in part resorbed and eliminated in the urine.
m, on the other hand, proposed the hypothesis that the sub-
e originates in a diseased or disordered liver, as bilirubin does
e same organ in health, and accordingly he regards the appear-
of much urobilin in the urine as evidence of hepatic insuf-
cy. Others, again, maintain that urobilin is formed in the
es at large either by the reduction of bilirubin or directly from
lood pigment. The first view is notably held by Kunkel, Mya,

Jaksch, Klinische Diagnostik, 4th ed., p. 339. Glinsky, Maly's Jahresber.,
vol. xxiii, p. 484. Langgaard, Virchow's Archiv, vol. lxxxvi. W. Hirs-
, Deutsch. Arch., 1899, vol. lxii, p. 53.

Giarré, and others, while the hematogenous theory is notably represented by Gerhardt. Garrod discusses these various hypotheses at some length in his most interesting lecture on the urinary pigments in their pathological aspects, in which he personally inclines to the intestinal theory, as now held by Müller, Schmidt, Esser, and others. In a work of this scope it would lead too far to discuss the various investigations which lend themselves in support of this view, and I can here quote only the following from Garrod's paper: "The chief seat of the formation of urobilin (for it is convenient to employ this term as including both pigment and chromogen) is undoubtedly the intestinal canal. This can only be gainsaid by denying the identity of the urinary and fecal pigments. The quantity normally present in the feces is far larger than that which enters the intestine with the bile (when a small amount is found), and there is strong evidence that the urobilin in bile is itself of intestinal origin. This being so, it is clear that theories other than the intestinal and its modifications merely attempt to trace a second source for the urobilin of the urine. It is equally clear that the substance from which the intestinal urobilin is formed is the bile pigment. Under ordinary conditions the bile pigment is destroyed in its passage along the intestine, and does not appear as such in the feces. In its place we find large quantities of urobilin, which in its turn disappears when occlusion of the common duct prevents the entrance of bile into the intestine. Again, when under certain morbid conditions the bile pigment passes along the intestine unaltered, urobilin is absent from the feces. However, the conversion of bilirubin into urobilin is no mere process of reduction, but involves a much more radical change, with elimination of nitrogen. That the change is brought about by bacterial action there is much evidence to show. When bile is inoculated with fecal material and kept in an incubator a formation of urobilin rapidly takes place, and at the same time the bile pigment diminishes, and ultimately disappears."

From its frequent occurrence in febrile urines pathological urobilin has also received the name *febrile* urobilin.

Its presence is very common in hepatic cirrhosis. In 12 cases of the atrophic and hypertrophic variety v. Jaksch was able to demonstrate urobilin in every instance, a point which may at times be of considerable diagnostic importance. I have observed urobilin in a few cases of hepatic cirrhosis, chronic malaria, and pernicious anemia, in all of which the skin presented a light icteric hue, and in which bile pigment was absent from the urine. Unfortunately, an examination of the blood was not made, and I have hence not been able to confirm the statement of v. Jaksch that bilirubin occurs in the blood in almost every case in which urobilin is present in the urine. Syllaba, however, has shown that in pernicious anemia, urobilinuria is quite constantly associated with bilirubinemia (see the latter). Urobilin has also been noted in cases of carcinoma, scurvy, Addi-on's disease, hemophilia, in cases of retro-uterine hematocele, in xtra-uterine pregnancy, following intracranial hemorrhages, etc. ccording to Bargellini, the degree of constipation in simple atony f the bowel is without influence upon the amount of urinary uro-ilin, but he states that in typhoid fever it causes an obvious increase; hereas disinfection or emptying of the large bowel produces a notable iminution in the amount. Urobilinuria, according to Samberger,[1] s common early in secondary syphilis and referable to increased estruction of red cells. In some cases the urobilinuria is only bserved after the mercurial treatment has been instituted, and sub-equently disappears.

Urines rich in urobilin usually present a dark-yellow color which s strongly suggestive of the presence of bilirubin; even the foam n such cases may be colored, making the resemblance between the wo pigments still more complete. This dark color, however, is not ue to urobilin, but to associated pigments.

GERHARDT'S TEST.—If the urine contains much urobilin, which he color will indicate, 10 to 20 c.c. are extracted with chloroform by haking, and the extract treated with a few drops of a dilute solu-ion of iodopotassic iodide. Upon the further addition of a dilute olution of sodium hydrate the chloroform extract is colored a yellow r yellowish brown, and exhibits a beautiful green fluorescence; this s even more intense than that noted in the case of normal urobilin.

BRAUNSTEIN'S TEST.—The reagent is composed of 100 c.c. of a con-entrated solution of copper sulphate, 6 c.c. of concentrated hydro-hloric acid, and 3 grams of ferric chloride; 20 c.c. of urine are reated with 3 to 4 c.c. of the reagent and shaken with chloroform. n the presence of urobilin a rose to a red color develops.

SCHLESINGER'S TEST.—10 c.c. of urine are treated with an equal uantity of a 1 per cent. solution of acetate of zinc in absolute alcohol. he mixture is agitated and filtered, when in the presence of urobilin he filtrate will show distinct fluorescence.

SPECTROSCOPIC EXAMINATION.—The urine is best examined as ollows: 50 c.c. of urine are extracted in a separating funnel with myl alcohol, which takes up both the pigment and its chromogen. fter standing for several hours the urine is allowed to flow away y opening the stopcock, when the alcoholic extract is decanted from bove, and is treated with a concentrated alcoholic and ammoniacal olution of zinc chloride. In the presence of urobilin the liquid hows a beautiful fluorescence, and on spectroscopic examination a ingle band of absorption is seen between *b* and *F*. In acid solu-ions, on the other hand, a single band is likewise obtained between *b* and *F*, but this extends to the right beyond *F*, and is much darker. Should the urine contain much urobilin, its special extraction is not necessary. In such an event the acid urine shows the acid spectrum, while the alkaline band is obtained after the addition of ammonia. (See also Bang's Test.)

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[1] Arch. f. Dermat. und Syph., 1903, vol. lxvii.

Literature.—A. E. Garrod, loc. cit. A. E. Garrod and F. G. Hopkins, "On Urobilin," Jour. of Physiol., 1898, vol. xxii, p. 451. Maly, Centralbl. f. d. med. Wiss., 1871, vol. ix, p. 849. Hayem, Gaz. hebdom., 1887, vol. xxiv, pp. 520 and 534; and Gaz. des hôp., 1889, vol. lxii, p. 1314. Kunkel, Virchow's Archiv, 1880, vol. lxxix, p. 655. Mya, Arch. ital. di clin. med., 1891, vol. xxx, p. 101; and Lo Sperimentale, 1896, vol. l, p. 71. Giarré, ibid., 1895, vol. xlix, p. 89, and 1896, vol. l, p. 81. F. Müller, Schlesische Gesellsch. f. vaterländ. Kultur, January, 1892. A. Schmidt, Verhandl. d. XIII Congress. f. inn. Med., 1895, p. 320. Esser, Untersuchungen über d. Entstehungsweise d. Hydrobilirubins, etc., Diss. Bonn., 1896. Bargellini, Lo Sperimentale, 1892, vol. xlvi, p. 119. v. Jaksch, Zeit. f. Heilk., 1895, vol. xvi, p. 48. D. Gerhardt, Zeit. f. klin. Med., 1897, vol. xxxii, p. 313.

Melanin and Melanogen.—In cases of melanotic disease it has been repeatedly observed that the urine, which usually and probably always presents a normal yellow color when voided, gradually becomes darker upon exposure to the air, and finally turns black. Such urines generally contain melanin and its chromogen in solution; deposits of melanin granules by themselves are only occasionally seen, and are not characteristic, as they may also be found in cases of chronic malarial intoxication, etc.

While the occurrence of melanin in the urine is probaly indicative in most cases of the existence of melanotic tumors, it should be stated that this symptom cannot be regarded as pathognomonic, as it may be absent in the case of melanotic tumors, and present in wasting diseases and inflammatory affections, and may at times, though very rarely, be associated with non-pigmented growths. Nevertheless, its occurrence should always be regarded with suspicion, and, taken in conjunction with other symptoms, will often lead to a correct diagnosis.

Tests for Melanin and Melanogen.—1. The presence of melanogen may be assumed if upon the addition of ferric chloride solution a black precipitate appears in the urine, which is soluble in a solution of sodium carbonate, and can be reprecipitated as a black or brownish-black powder by mineral acids. Instead of ferric chloride barium hydrate may also be used.

2. A few cubic centimeters of urine are treated with bromine-water, when in the presence of melanin or melanogen a precipitate is obtained, which is yellow at first, but gradually turns black.

Literature.—T. H. Eiselt, "Die Diagnose d. Pigmentkrebses durch d. Harn," Prag. Vierteljahrschr. f. praktische Heilk., 1858, iii, p. 190, and 1862, vol. iv, p. 26. Senator, "Ueber schwarzen Urin," Charité Annal., 1891. Hoppe-Seyler, Zeit. f. physiol. Chem., 1891, vol. xv, p. 179. F. Grohe, "Zur Gesch. d. Melanaemie," Virchow's Archiv, 1861, vol. xx, p. 306.

Phenol.—Phenol, according to Brieger, occurs only in very small amounts in human urine, the usual phenol reactions being largely referable to paracresol. Normally, about 0.03 gram is eliminated in the twenty-four hours, but in pathological conditions much larger quantities may be found. Remembering the origin of phenol, it is clear that an increased elimination may be observed whenever putrefactive processes are going on in the tissues and cavities of the body, or whenever there is an increase in the degree of intestinal putrefaction, though in the latter condition the indican is usually the only conjugate sulphate that is found increased. In peritonitis, diphtheria, erysipelas, scarlatina, empyema, pulmonary gangrene, putrid bronchitis, etc., an increased elimination of phenol is commonly seen, as also in certain cases of pernicious vomiting of pregnancy. Important from a diagnostic standpoint, further, is the fact that in uncomplicated cases of typhoid fever no increase is observed, while this is common in tuberculous meningitis.[1] The largest amounts, of course, are seen in cases of poisoning with carbolic acid or one of its derivatives (hydroquinone, pyrocatechin, salicylic acid), where the urine may darken on standing, thus simulating true melanuria.

As the quantitative estimation of phenol is too complicated for the purposes of the general practitioner, Salkowski's qualitative test is here also described. From the intensity of the reaction certain conclusions may be drawn as to the amount present. It is especially serviceable in cases of suspected poisoning with carbolic acid.

SALKOWSKI'S TEST.—About 10 c.c. of urine are boiled in a test-tube with a few cubic centimeters of nitric acid, and, on cooling, treated with bromine-water. The development of a pronounced turbidity or the occurrence of a precipitate indicates the presence of an increased amount of phenol.

QUANTITATIVE ESTIMATION. *Principle.*—When potassium-phenyl sulphate is treated with hydrochloric acid, phenyl sulphate results, which further takes up one molecule of water, giving rise to the formation of sulphuric acid and phenol.

From the action of bromine-water upon phenol a yellowish-white crystalline precipitae of tribromophenol results:

$$C_6H_5.OH + 6Br = 3HBr + C_6H_2Br3.OH.$$

As 331 (molecular weight) parts by weight of tribromophenol correspond to 94 (molecular weight) parts by weight of phenol, the amount of the latter contained in a certain volume of urine is readily determined according to the equation

$$331:94::x:y; \text{ and } y = \frac{94.x}{331} = 0.28398\,x,$$

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[1] A. Strasser, "Ueber d. Phenolausscheidung bei Krankheiten," Zeit. f. klin. Med., vol. xxiv, p. 543. Brieger, Zeit. f. klin. Med., 1881, vol. iii, p. 468. Kast u. Baas, Münch. med. Woch., 1888, vol. xxxv, p. 55.

in which x indicates the weight of the tribromophenol found in the amount of urine employed, and y the corresponding quantity of phenol.

METHOD.—From 500 to 1000 c.c. of urine are treated with one-fifth of an equivalent amount of dilute hydrochloric acid (1 to 4), and distilled so long as a specimen of the distillate is rendered cloudy upon the addition of bromine-water (1 to 30), the specimens used for this purpose being carefully preserved. The total quantity of the filtered distillate, together with the specimens which have been set aside, is now treated with bromine-water, shaking the mixture after each addition of the reagent until a permanent yellow color results. Beyond this point further addition is beset with danger, as compounds will be formed which contain more bromine, the presence of which would indicate a smaller amount of phenol than that actually contained in the urine. After two or three days the precipitate is collected on a filter which has been dried over sulphuric acid, washed with water containing a trace of bromine, and then dried over sulphuric acid and weighed.

Salol and *salicylic acid* may be recognized from the fact that such urines when treated with a solution of ferric chloride develop a marked violet color which does not disappear on standing. The reaction thus differs from that obtained with diacetic acid.

Alkapton.—Urines are at times, though very rarely, seen which, like the phenol urines, turn dark on standing, but in which the change in color is neither referable to the presence of phenol or its derivatives, nor to melanin. Such urines are of a normal color when passed, but gradually turn reddish brown upon exposure to the air. Treated with a small amount of alkai, this change occurs almost immediately. Fehling's solution is reduced on the application of heat, while bismuth is not affected. Ammoniacal silver solution is reduced in the cold, and a temporary bluish-green color develops when the urine is treated with a ferric salt. The fermentation test is negative, and examination with the polarimeter shows that the substance in question is not glucose. With phenylhydrazin no osazone is formed.

Bödeker, who first observed a urine of this kind, termed the substance giving rise to the reactions just described alkapton, and subsequently expressed the belief that his alkapton might possibly have been pyrocatechin. Subsequent investigators succeeded in isolating substances from such urines which have been variously termed pyrocatechuic acid, urrhodinic acid, glucosuric acid, uroleucinic acid, and uroxanthinic acid. Baumann and Wolkow later were able to isolate *homogentisinic acid* in pure form from the urine of such cases, and expressed the belief that some of the substances obtained by previous observers were in reality the same. Since that time this acid has also been found by Garrod, Ogden, Stange, Stier, and others.

Of the origin of alkapton little is known. Baumann expressed the opinion, that homogentisinic acid might be derived from tyrosin, and that the condition is referable to the activity of special microorganisms in the upper portions of the intestines. As a matter of fact the amount of homogentisinic acid can be materially increased by the administration of tyrosin, and Mittelbach has shown that if the substance is given in frequently repeated and small doses almost the entire amount reappears in the urine as homogentisinic acid. Tyrosin, however, belongs to the *para*-series, while homogentisinic acid is an *ortho*-compound, so that the transformation of tyrosin into homogentisinic acid cannot be a direct process, and it has accordingly been questioned whether Baumann's view regarding the origin of alkapton is correct. There is evidence indeed to show that homogentisinic acid does not originate in the intestines, viz., is not a product of bacterial activity. It has thus been found that the alkaptonuria does not cease during starvation, and that a restriction of the putrefactive processes in the intestines by means of oil of turpentine, a kefir diet, and the administration of β-naphthol does not lead to a diminished elimination of homogentisinic acid. It has never been found in the feces, moreover, and Garrod has shown that after inoculation of common bouillon, meat juice, or tyrosin broth with alkaptonuric feces homogentisinic acid is not formed. On the other hand, Embden observed that when an alkaptonuric individual took homogentisinic acid by the mouth a far larger portion appeared in the urine than when the same substance was administered to a healthy individual, which suggests that the alkaptonuria may be referable to impairment of the normal processes of oxidation. Very significant is the discovery that a notable increase follows the administration of phenylalanin, and that the ingestion of phenylacetic acid will increase the power of reduction and of rotation of the urine. Phenylpropionic acid and benzoic acid cause no increase in the elimination of homogentisinic acid.

The prevailing view is that alkaptonuria is a metabolic anomaly comparable to glucosuria and cystinuria; but, unlike glucosuria, it can scarcely be regarded as an expression of a pathological process. It may, of course, occur in individuals, suffering from disease, and has been observed in connection with glucosuria, in acute gastrointestinal catarrh, in phthisis, acute miliary tuberculosis, in one case of brain tumor, carcinoma of the prostate, etc. More frequently the condition is accidentally discovered in apparently healthy individuals, and has repeatedly been confounded with glucosuria owing to the positive reduction test with Fehling's solution.

Garrod, from an analysis of all the reported cases, concludes that the condition is nearly always congenital. In 32 known instances which were presumably congenital, 19 occurred in seven families. One family contained 4 alkaptonurics, three others 3, and the remaining three 2 each. In fully 60 per cent. of the cases, it appears from Garrod's studies, the parents of alkaptonurics were first cousins. There is thus far only one known instance in which the anomaly has been transmitted by an alkaptonuric father to his son.

The condition commonly persists through years and perhaps a lifetime. It may also occur as a transitory abnormality, however, as is apparent from the case of Hirsch, in which the condition persisted for three days, and the case of Geyger, in which the alkaptonuria was observed on only two days. A few observers further report the occurrence of alkaptonuria shortly preceding death.

Very interesting in this connection is the observation of Osler and others that the urine of patients with ochronosis will darken on standing and may contain homogentisinic acid. The pigmentation of the cartilages thus seemed to be a possible morphological expression of the urinary abnormality. But as Garrod has already stated, it is possible also that other substances besides homogentisinic acid may cause the blackening of the urine in ochronosis.

The amount of homogentisinic acid eliminated in the twenty-four hours is variable, but usually large. Baumann found an average elimination of 4.6 grams; the largest amount in twenty-four hours was 6 grams. In Meyer's case, a child one and one-half years old, 3.3 grams were passed pro die. Larger quantities are obtained after a liberal diet of meats than with a vegetable diet.

Isolation and Estimation (Garrod's Method).—The urine is heated nearly to boiling without any preliminary treatment, and for each 100 c.c. at least 5 or 6 grams of solid neutral lead acetate are added.

As soon as the acetate is dissolved, the bulky gray precipitate which forms is removed by filtration, and the filtrate, which has a pale-yellow color, is allowed to stand for twenty-four hours in a cool place. If the urine be very rich in homogentisinic acid, or if the flask containing the filtrate be placed upon ice, minute acicular crystals, which are almost colorless, quickly form; but as a rule crystallization does not commence until several hours have elapsed. The crystals are then much larger, are grouped in stars or rosettes, and are more deeply colored.

In summer weather it would probably be desirable to start the crystallization by artificial cooling; but although the process is greatly accelerated at a low temperature, the total yield is not materially increased.

If the formation of the crystals be long delayed, the liquid may be warmed again and more lead acetate added.

After the lapse of twenty-four hours crystals cease to form, even when the liquid is placed upon ice.

The crystalline product so obtained is lead homogentisinate. When the crystals are dissolved in hot water the solution assumes a deep-brown color with alkalies; it reduces Fehling's solution readily with the aid of heat, and yields a transitory deep-blue color with a dilute solution of ferric chloride. From the lead salt free homogentisinic acid may be obtained by decomposing it with hydrogen sulphide.

For clinical purposes the following method also may be employed:

BAUMANN'S METHOD.—50 c.c. of urine are treated with 15 grams of ammonium chloride, which should be brought into solution by shaking, in a stoppered graduate. After standing for about twelve hours to allow the uric acid to separate out the solution is filtered and an accurately measured portion of the filtrate titrated with a decinormal ammoniacal solution of silver nitrate. The titration is continued until a further reduction of the silver solution does not occur, which is ascertained by acidifying a few drops of the filtered mixture with hydrochloric acid, when in the presence of free silver a turbidity referable to silver chloride occurs. Accuracy within narrower limits than $\frac{1}{4}$ c.c. is scarcely possible, as the turbidity referable to silver chloride can only be recognized within 0.2 to 0.3 c.c. According to Baumann, 240 to 245 c.c. of the silver solution represent 1 gram of homogentisinic acid.

LITERATURE.—Bödeker, Annal. d. Chemie u. Pharmakol., 1861, vol. cxvii, p. 98. Baumann u. Wolkow, Zeit. f. physiol. Chem., 1891, vol. xv, p. 228. Stier, Berlin. klin. Woch., 1898, vol. xxxv, p. 185. Embden, Zeit. f. physiol. Chem., 1893, vol. xvii, p. 182, and vol. xviii, p. 304. Ogden, Zeit. f. physiol. Chem., 1895, vol. xx, p. 280. Futcher, N. Y. Med. Jour., 1898, vol. lxvii, p. 69. Garrod, Jour. Physiol., 1899, vol. xxiii, p. 512; and Med.-Chir. Trans. Royal Soc., vol. lxxxii, p. 367. E. Meyer, Deutsch. Arch., vol. lxx, Heft 5 u. 6. F. Wittelbach, ibid., 1901, vol. lxxi, p. 50.

Blue Urines.—Blue urines are sometimes seen, the color of which is due to indigo formed from urinary indican within the urinary passages. Their occurrence can only be regarded as a medical curiosity. One case of this kind is reported by McPhedran and Goldie,[1] in which after direct extraction of the urine with ether only a faint reaction was obtained on further examination, and which probably was referable to incomplete previous extraction. Formerly, when indigo was employed in the treatment of epilepsy, blue urines were frequently seen. At the present time, when methylene blue is occasionally used in the treatment of malaria and chyluria, this pigment is found in the urine.

Green Urines.—Green urines have also been described; the cause of the color, however, has not been ascertained.

Pigments referable to Drugs.—Certain drugs may also cause changes in the normal color of urine, and in doubtful cases inquiry in this direction should be made. It has been pointed out that carbolic acid, hydroquinone, pyrocatechin, and salol cause the appearance of a dark-brown color, and that after the administration of indigo and methylene blue blue urines are voided. Santonin, rheum, and senna color urines a bright yellow, so that they may resemble icteric urines. The yellow color in such cases is changed to an intense red by the addition of an alkali, and, if ammoniacal fermentation is going on at the same time in the bladder, the patient may believe himself to be suffering from hematuria. The red color thus produced is due to the action of the alkali upon chrysophanic acid. When urines containing copaiba are treated with hydrochloric acid a red color results, which changes to violet upon the application of heat. During the administration of potassium iodide, or the use of iodine in any form, a dark mahogany color is obtained when the urine is treated with nitric acid. In doubtful cases Stokvis' modification of Jaffé's test for indican should be employed, when in the presence of an iodide the chloroform assumes a beautiful rose-red color.

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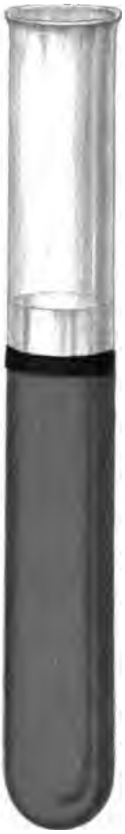
[1] Transactions Association American Physicians, 1901.

For the detection of other drugs and poisons in the urine the reader is referred to special works.

Ehrlich's Diazo Reaction.—Under certain pathological conditions, and especially in typhoid fever, a chromogen may be present in the urine, which, when treated with diazo-benzene-sulphonic acid and ammonia, imparts a red color to the urine, varying from eosin to a deep garnet red. This reaction, which is generally spoken of as Ehrlich's reaction, or the *diazo reaction*, was at one time regarded as pathognomonic of typhoid fever. Subsequent examinations, however, have shown that it may also be present in other diseases. Michaelis, who has made an exhaustive study of this question, divides into four groups the diseases in which the reaction has been observed. In the first group, comprising diseases of the nervous system, chronic diseases of the heart and kidneys, malignant tumors, etc., the reaction is rarely seen. When present, it usually indicates a secondary infection. The second group includes those diseases in which the reaction is almost always present, namely, typhoid fever and measles. In the diseases of the third group it is often, though not invariably, observed. Under this heading are classed scarlet fever, erysipelas, pneumonia, diphtheria, pyemia, acute miliary tuberculosis, etc. The fourth group comprises pulmonary tuberculosis, and includes acute caseous pneumonia.

The value of Ehrlich's reaction in typhoid fever was at first overestimated, but is at present certainly underestimated. I have studied this problem with great care, and after many years' experience maintain, as I did years ago, that the test is a most important diagnostic aid in the disease in question. As a general rule the reaction is present as early as the fifth or sixth day, and may persist into the third week; it then disappears, but may reappear when a relapse occurs. This fact is generally overlooked and should be borne in mind in the differential diagnosis from acute tuberculosis. Excepting in children, its absence from the fifth to the ninth day usually indicates

PLATE XIX



Ehrlich's Diazo-reaction, as modified by the author. The orange color in the lower portion of the test tube may be obtained in any urine; the dark carmine ring indicates the presence of the reaction in a well-pronounced degree, the colorless zone above is intended to indicate the ammonia that has been added.

a mild case. This rule, however, is not without exception. When the reaction is continuously present after the third week I am inclined to suspect acute tuberculosis. It may be present as early as the fourth day of the disease.

In paratyphoid, as in typhoid fever, the reaction is also fairly constant.

Of late much attention has been paid to the occurrence of Ehrlich's reaction in pulmonary phthisis. As a result of his investigations Michaelis concludes that its presence in such cases indicates either that the process is very extensive or that it will progress very rapidly, and that the prognosis is grave. A cure, be believes, is impossible, and improvement, if any, only temporary. Clemens notes that of 100 cases of phthisis which ended fatally 87 showed the diazo reaction; Rütimeyer obtained positive results in 85 cases out of 106 which died. Of 13 cases of acute tuberculous pneumonia Fränkel and Troje found a positive reaction in 11. Grundriss states that in his fatal cases the reaction was present without exception. Similar results have been obtained by Cnopf, Sée, Goldschmidt, and others. Michaelis himself reports that of 111 cases of phthisis which were admitted to the Berlin Charité with well-marked reaction 80 died in the hospital, 13 were discharged unimproved, 3 were transferred to other hospitals, and 15 left improved. In other words, of these 111 cases a fatal result was known to have occurred in 72 per cent. Stadelmann states that of 38 other cases with positive reaction 28 died in the hospital—*i. e.*, about 75 per cent. The subsequent fate of the remaining cases was not ascertained; but we may well assume that of these at least 50 per cent. died; so that we may formulate the general rule that a fatal result may be anticipated in about 85 per cent. of all cases of phthisis in which a positive reaction is obtained. Michaelis, moreover, suggests that the end may be expected to occur within six months from the time at which a *persistent* Ehrlich reaction is established. Exceptions occur, but the above is the rule. In Koch's institute at Berlin patients presenting the diazo reaction are not treated with tuberculin (Brieger).[1]

In tuberculous peritonitis the diazo reaction is found in about 25 per cent. of all cases.

As regards the frequency of occurrence of the reaction in diphtheria, it appears from the observations of Rivier[2] and others that it is decidely uncommon. Of his own 118 cases, and 44 additional ones collected from the literature, only 10 gave a positive result; and of these, 4 should be eliminated as they occurred in complicated cases; so that the reaction was absent in about 97 per cent.

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[1] Discussion on Tuberculosis, Michaelis, Deutsch. med. Woch., 1901, vol. v, p. 211.

[2] Thèse de Paris, 1898.

In the scarlatiniform erythema due to serum treatment the reaction is absent, while in true scarlatina it is fairly common. Including a number of cases collected from the literature Rivier found a positive reaction in 41 cases out of 73. He concludes that in the differential diagnosis between the two conditions scarlatina may be affirmed if the reaction is positive, while if negative there is strong presumptive evidence against the disease.

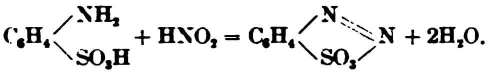
In measles a positive reaction was obtained in 75 of 85 cases.

Rüttimeyer obtained the reaction in pulmonary actinomycosis.

The reaction has been referred to the presence of alloxyproteinic acid,[1] but this is denied by Clemens.

As the preparation of chemically pure, crystalline diazo compounds is a difficult process, Ehrlich uses sulphanilic acid, which, when treated with nitrous acid in a nascent state, gives rise to the formation of diazo-benzene-sulphonic acid, as is shown by the equations:

1. $NaNO_2 + HCl = NaCl + HNO_2$.

2. 

$$C_6H_4\begin{cases}NH_2\\SO_3H\end{cases} + HNO_2 = C_6H_4\begin{cases}N{=}N\\SO_3\end{cases} + 2H_2O.$$

Para-amino-benzene-sulphonic acid. Diazo-benzene-sulphonic acid.

This is the active principle in the mixture employed.

Other compounds may, of course, also be used, such as meta-amino-benzene-sulphonic acid, ortho- and para-toluidin-sulphonic acid, etc.; but of all these, Ehrlich found the common sulphanilic acid the most convenient. Two solutions, which must be kept in separate bottles, are employed. The one is a 5 per cent. solution of hydrochloric acid, to which sulphanilic acid is added in the proportion of 1 gram for every 100 c.c. The other is a 0.5 per cent. solution of sodium nitrite.

The two solutions are mixed in the proportion of 40 to 1 immediately before using. A few cubic centimeters of urine are then treated with an equal volume of the reagent; the mixture is shaken and rendered alkaline with ammonium hydrate. This is best allowed to flow down the sides of the tube, so as to form a layer above the mixture. At the junction of the two fluids a colored ring will now be observed. With urines which do not contain the chromogen this will be a more or less distinct orange, while in its presence a red color is obtained. The intensity of this color may vary from eosin to a deep garnet red. If the mixture is now agitated and the reaction is positive, the foam will likewise be colored red, and upon pouring the solution into a porcelain basin containing ch water a beautiful salmon color is obtained, even if only traces the chromogen are present. Carried out in this manner no ques- n will arise as to the presence or absence of the reaction. Ehr- h states that on standing a green sediment forms in the alkalinized xture, and he regards this sediment as especially characteristic. y experience has been that this becomes manifest only when the lor reaction is well pronounced, and I am inclined to attach more mportance to the salmon color obtained upon copious dilution. With normal urines this is never obtained, and it can still be een when inspection of the fluid in the test-tube would leave in doubt.

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[1] Bondzynski u. Panek, Berlin. d. deutsch. chem. Ges., 1903, vol. xxxv, p. 2951.

The older method of Ehrlich I have abandoned, as the test just described is simpler, and, in my experience, just as reliable. He advised the addition of about 50 c.c. of absolute alcohol to 10 c.c. of urine, subsequent filtration, and examination of the filtrate, as just described.

Greene states that if 1 part of the sodium nitrite solution is added to 100 instead of 40 parts of the sulphanilic acid solution, a positive reaction is no longer obtained in cases of croupous pneumonia and of pulmonary tuberculosis, while in typhoid fever the reaction occurs with the same intensity.

While in the absence of the chromogen, as I have already stated, a more or less pronounced orange color is usually obtained, exceptions have been noted. Ehrlich thus records that in urines containing biliary coloring matter an intensely dark, cloudy discoloration occurs at times, which upon boiling is changed to a well-marked reddish violet. In rare instances of ulcerative endocarditis, hepatic abscess, and intermittent fever, and more commonly in pneumonia about the time of the crisis, Ehrlich further observed an intense yolk-yellow color, before the addition of the ammonia, which becomes somewhat lighter after this is added. The reaction is supposedly referable to urobilinogen (*egg-yellow reaction*).

Of interest is the observation of Burghart, that after the administration of tannic acid, gallic acid, and certain iodine preparations, Ehrlich's reaction disappears from the urine. But, as Burghart himself suggests, it is likely that this inhibitory effect is not exerted upon the diazo-forming substance, but upon the reagents employed. Other factors, which may prevent the occurrence of Ehrlich's reaction, in pulmonary tuberculosis at least, are the occurrence of renal complications (albuminuria). Naphthalin, after its administration by the mouth, according to my experience may cause a reaction, the color of which corresponds exactly to that of the diazo reaction.

Other observers have noted a similar reaction after the administration of opium (morphine, heroine), alcohol in large amount, phenol, cresol, creosote, and guaiacol. Golden, on the other hand, denies its occurrence after the use of some of the substances mentioned.

LITERATURE.—Ehrlich, Zeit. f. klin. Med., 1882, vol. v, p. 285; Charit. Annal., 1883, vol. viii, p. 28, and 1886, vol. xi. p. 139. Goldschmidt, Münch. med. Woch., 1886, vol. xxxiii, p. 35. Rütimeyer, Corresp. Blatt. f. Schweizer Aerzte, 1890, vol. xxvi. Greene, Med. Record, Nov. 14, 1896. C. E. Simon, Johns Hopkins Hosp. Bull., 1890. J. Friedenwald, N. Y. Med. Jour., 1893. M. Michaelis, Berlin. klin. Woch., 1900, p. 274; and Deutsch. med. Woch., 1899, p. 156. J. R. Arneill, Amer. Jour. Med. Sci., 1900, p. 296.

Ehrlich's Dimethylaminobenzaldehdye Reaction.—Ehrlich has shown that under various pathological conditions a fine cherry-red color develops on shaking a specimen of urine with a few drops of dimethylaminobenzaldehyde in acid solution, and that the resulting pigment can be in part extracted with chloroform, and almost entirely so with epi- or dichlorhydrin. With normal urines a similar reaction can be obtained, but it is much less intense, and if done at ordinary temperatures a distinct red color does not develop. On heating, however, it appears, and can likewise be extracted with epichlorhydrin. The reaction, according to O. Neubauer, is due to urobilinogen.

As regards the occurrence of the reaction in disease I can summarize my results as follows: (1) A direct reaction, of pathological grade, does not occur in health. (2) A positive reaction is most commonly obtained in cases of tuberculosis. (3) It may also be seen in non-tuberculous cases, both febrile and non-febrile. (4) It is not dependent upon the presence of the body which gives rise to the diazo reaction. (5) For its production elevation of temperature, gastro-intestinal disturbances, and cyanosis are not essential. (6) Common to all cases seems to be an increased katabolism of the tissue albumins.

My positive results include cases of pulmonary tuberculosis, tuberculosis of the hip-joint, pneumonia, typhoid fever, appendicitis, embarras gastrique, icterus, malignant endocarditis, empyema, esophageal carcinoma, and a remarkable instance of traumatic neurosis, in which a loss of weight of from sixty to seventy-five pounds had occurred.

My list of negative cases, on the other hand, includes, first of all, a large number of normal or supposedly normal individuals; in addition, cases of normal labor, neurasthenia, hysteria, diabetes, aortic aneurysm, myelogenous leukemia, lymphatic leukemia, acute nephritis (scarlatinal), simple diarrhea, morphinism, valvular disease, phthisis (stationary), diphtheria (before and after the use of antitoxin), typhoid fever, cases of abortion, appendicitis, influenza, chronic nephritis, cystitis, pyelitis (calculous), measles, tuberculosis of the hip-joint, cystic kidney, carcinoma of the kidney, tonsillitis, acute and chronic bronchitis, pneumonia, icterus, tuberculous peritonitis, general erythema; varicocele; following various operations, such as nephrorrhaphy, removal of pus tubes, operations for vesicovaginal fistula, fistula in ano, and suspension of the uterus. Examination of a urine containing cystin and diamins was also negative. A comparison of the negative with the positive cases will show at once that not all cases of pulmonary tuberculosis, tuberculous hip-joint disease, pneumonia, typhoid fever, appendicitis, and icterus give a positive result. So far as tuberculosis is concerned, however, it appears that the reaction is more likely to occur in the actively progressive cases than in those which are more or less stationary. It was also noted that the positive cases almost all gave a positive diazo reaction, while in the negative cases this was not obtained. Exceptions, however, may also occur.

In my personal examinations I employed a 2 per cent. solution of dimethylparaminobenzaldehyde in equal parts of water and concentrated hydrochloric acid. A few cubic centimeters of urine in a test-tube are treated with from 5 to 10 drops of the reagent; the mixture is set aside or agitated for a few minutes and the color then noted. Normal urines usually turn a greenish yellow, or the normal color merely becomes intensified. At times a dark-amber color develops, though this is less common in health, unless the urine is brought to the boil before the reagent is added. In this way it is a common experience to meet with moderate or dark-amber tints. With these reactions,. however, I have not occupied myself, and, like Clemens and Koziczkowsky, I have only noted the reaction as positive when a distinct *cherry-red* color developed, either immediately on adding the reagent or after agitation or standing.

LITERATURE.—Ehrlich, med. Woch., 1901, No. 15. Clemens, Deutsch. Arch., 1901, vol. lxxi, p. 168. Koziczkowsky, Berl. med. Woch., 1902, vol. xxxix, No. 44. Simon, Amer. Jour. Med. Sci., 1903, vol. cxxvi, p. 471.

Acetone.

The amount of acetone which may be found in the urine under normal conditions varies between 0.008 and 0.027 gram, and is greatly influenced by the character of the diet. Whenever the carbohydrates are withdrawn the quantity rapidly increases and reaches its maximum about the seventh or eighth day. At this time from 200 to 700 mgrms. may be eliminated in the twenty-four hours. If, then, carbohydrates are again added to the diet, the acetonuria soon disappears. This result is not reached, however, if fats are substituted for the carbohydrates. The acetonuria is greatest when but little albuminous food and no carbohydrates at all are ingested, and during starvation the same amounts are essentially found. Increased amounts are found in fevers, the various cachexias, in conditions associated with inanition, etc.[1] The source of the acetone in these cases was formerly sought in the increased albuminous destruction, but according to more recent research it appears that in some manner the fat metabolism is involved and that the acetonuria is the result.

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[1] v. Jaksch, Ueber Acetonurie u. Diaceturie, Hirschwald, Berlin, 1885. Rosenfeld, Centralbl. f. inn. Med., 1895, vol. xv. Waldvogel, "Zur Lehre von der Acetonurie," Zeit. f. klin. Med., vol. xxxviii, p. 506.

Most important is the diabetic form of acetonuria. It may be stated, as a general rule, that the diagnosis of diabetes mellitus is justifiable whenever sugar and notable quantities of acetone are found in the urine. The amount of acetone, moreover, stands in a direct relation to the intensity of the disease, the maximum excretion being usually observed toward the fatal end.[1] Whether or not this form of acetonuria can always be explained upon the basis given above remains an open question. There can be no doubt, however, that the threatening symptoms which are so commonly associated with a greatly increased elimination of acetone will often disappear, at least temporarily, if carbohydrates are administered in large amounts. It is certain, moreover, that diabetic coma is more apt to occur when the old-fashioned plan of excluding carbohydrates entirely from the dietary of diabetic patients is adopted. Hirschfeld[2] suggests that in every case of diabetes the excretion of acetone be carefully followed, and that large amounts of carbohydrates be administered whenever the acetonuria approaches a dangerous extent.

Of the febrile diseases in which acetonuria has been observed may be mentioned typhoid fever, pneumonia, scarlatina, measles, acute miliary tuberculosis, acute articular rheumatism, and septicemia. In those of short duration, on the other hand, even if the fever is high, as in acute tonsillitis, intermittent fever, the hectic fever of phthisis, etc., an increased elimination of acetone is rarely observed. In the continued fevers the acetonuria is largely referable to the character of the diet, as carbohydrates are usually excluded entirely, and I have repeatedly observed that a return to the normal occurred as soon as sugar was administered in amounts varying from 50 to 100 grams.

In certain nervous and mental diseases, as in general paresis, melancholia, following epileptic seizures, and in tabes, acetonuria is frequently observed. During the second stage of general paresis increased amounts are indeed quite constantly found, but Hirschfeld is probably correct in stating that the psychotic form of acetonuria is largely referable to improper feeding.

A notable degree of acetonuria has been observed in connection with the pernicious vomiting of pregnancy,[3] and in eclampsia (Baginski). A certain amount of acetone occurs normally during the first two days of the puerperal period, but usually disappears by the third day.

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[1] v. Jaksch, Zeit. f. klin. Med., 1885, vol. x, p. 362. Lorenz, ibid., 1891, vol. xix, p. 19.

[2] Beobachtungen über d. Acetonurie u. das Coma diabeticum, Zeit. f. klin. Med., vol. xxviii, p. 176, and vol. xxxi, p. 212.

[3] H. Baldwin, Amer. Jour., Oct. 1905, p. 649.

According to Vicarelli[1] acetonuria occurring in the course of pregancy is evidence of the death of the fetus. This is possibly the rule, but exceptions have been observed.

In the primary diseases of the stomach, and notably in carcinoma, cetonuria is frequently observed, and it is possible that the acetone n these cases is, to some extent at least, formed in that organ directly rom the proteids ingested. The facts that in carcinoma acetone may be observed at a time when marked loss of flesh has not as yet occurred, nd that larger amounts of acetone may be found in the stomach han in the urine, are certainly in favor of this view.[2]

An enterogenic form of acetonuria has further been described, and t has been urged that in these cases the acetone is referable to the ormation of unusually large amounts of fatty acids. Acetonuria of this type is also observed following the ingestion of fatty acids as such (alimentary form).[3]

Acetonuria has further been observed early in the course of acute phosphorus poisoning, and may persist throughout, apparently without being an index of the severity of the case.

After chloroform narcosis the condition is also not uncommon.

Tests for Acetone. **Legal's Test.**[4]—This test may be applied to he freshly voided urine, but is not conclusive. Several cubic centimeters of urine are treated with a few drops of a strong solution of odium nitroprusside and sodium hydrate; the mixture assumes a red color, which rapidly disappears, and in the presence of acetone is replaced by a purple or violet red when acetic acid is added. As a rule, it is better to distil the urine (500 to 1000 c.c.) after the addiion of a little phosphoric acid (1 gram pro liter), and to employ the irst 10 to 30 c.c. of the distillate for one or more of the following ests.

Lieben's Test.[5]—A few cubic centimeters of the distillate are rendered strongly alkaline with caustic soda solution and treated with several drops of a dilute solution of iodopotassic iodide, when n the presence even of traces of acetone a precipitation of iodoform n crystalline form occurs. This may be recognized by its odor when the solution is heated, as also by the form of the crystals, which occur as hexagonal or stellate platelets. If traces of acetone only are present it is necessary to let the solution stand for a number of hours before examining.

Alcohol and acetic aldehyde give the same reaction. For this reason *Dunning's modification*[1] is sometimes to be preferred, although it is not as delicate. To this end a small amount of Lugol's solution is added to the distillate and a sufficient amount of ammonia to produce a black precipitate (nitrogen iodide). This disappears on standing and in the presence of acetone is replaced by iodoform.

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[1] Prager med. Woch., 1893, Bd. xxxiii und xxxv; also Knapp, Centralbl. f. Gynäk, 1897.

[2] H. Lorenz, loc. cit.

[3] Waldvogel u. Hagenberg, "Ueber alimentäre Acetonurie," Zeit. f. klin. Med., 900, vol. xiii, p. 443.

[4] Le Nobel, Arch. f. exper. Path. u. Pharmakol., 1884, vol. xviii, p. 9.

[5] Taniguti u. Salkowski, Zeit. f. physiol. Chem., 1890, vol. xiv, p. 476.

Gunning's test, like that of Legal, may be tried with the native urine first.

Frommer's Test.[2]—This test also may be applied directly to the urine, and is said to indicate the presence of 0.000001 acetone in 8 c.c. of water. It does not react with diacetic acid.

About 10 c.c. of urine are treated with about 1 gram of caustic soda in substance and—without waiting for the dissolution of the soda to occur—with 10 to 12 drops of an alcoholic solution of salicylic aldehyde (1 gram to 10 c.c. of absolute alcohol). The mixture is heated to 70° C. In the presence of acetone a marked purple-red color results at the zone of contact with the alkali.

If the alkali is added in solution the fluid first becomes yellow, later reddish, then purplish red, and finally dark carmine red. The color change occurs more rapidly by heating.

Denigès' Test (as Modified by Oppenheimer).[3]—The reagent is prepared as follows: 20 grams of concentrated sulphuric acid are poured into 100 c.c. of distilled water, when 5 grams of freshly prepared yellow mercuric oxide are added. The mixture is allowed to stand for twenty-four hours and is then ready for use.

This reagent is added to about 3 c.c. of urine, drop by drop, until the precipitate which is thus formed no longer disappears on stirring. When this point is reached a few more drops are added. After two or three minutes the precipitate is filtered off. The clear filtrate is further treated with about 2 c.c. of the reagent and 3 to 4 c.c. of a 30 per cent. solution of sulphuric acid, and boiled for a minute or two, or, still better, placed in a vessel with boiling water. In the presence of an abundant amount of acetone a copious white precipitate forms immediately; while in the presence of traces only (less than 1 to 50,000), a slight cloud develops on standing for several minutes. The precipitate is almost entirely soluble in an excess of hydrochloric acid.

If albumin is present, the urine becomes turbid at once when the reagent is added. In that case the test is continued as described, attention being directed to the coarser precipitate which occurs later. To such urines large amounts of the reagent must be added, the idea being to precipitate everything that can be precipitated with the reagent, before heating.

Oppenheimer claims that the test is as delicate as that of Lieben, giving a well-pronounced reaction with a dilution of 1 to 20,000, being still discernible with a dilution of 1 to 60,000. As diacetic d yields acetone when treated with mineral acids, a positive result always obtained when this is present. But as diacetic acid is ıally found only in association with acetone, this fact does not sen the value of the test, and is an error, moreover, which is common the other tests as well.

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[1] Jour. de pharmacol. et de chim., 1881, vol. iv, p. 30.

[2] Berlin. klin. Woch., Aug. 7, 1905, p. 1008.

[3] Ibid., 1899, p. 828.

Quantitative Estimation of Acetone.—For the purpose of estimat- the amount of acetone the method of Messinger, as modified by ippert, is now employed, and is greatly to be preferred to the older cedure of v. Jaksch.[1]

Principle.—The method is based upon the observation of Lieben t acetone gives rise to the formation of iodoform when treated h iodine in an alkaline solution. If then a solution of acetone is ated with a known amount of iodine, it is a simple matter to ermine the quantity present by retitrating the iodine which was used in the formation of iodoform.

Solutions required:

1. Acetic acid (50 per cent. solution).
2. Sulphuric acid (12 per cent. solution).
3. Sodium hydrate solution (50 per cent.).
4. A decinormal solution of iodine.
5. A decinormal solution of sodium thiosulphate.
6. Starch solution (see Boas' method of estimating lactic acid).

Preparation of the solutions:

1. The decinormal solution of iodine is prepared as described ewhere (see Boas' method of estimating lactic acid).

2. As the molecular weight of sodium thiosulphate—$Na_2S_2O_3$.- $_2$O—is 248, a decinormal solution of the salt would contain 24.8 ms to the liter. This quantity is dissolved in about 950 c.c. distilled water and brought to the proper strength by titration h a decinormal solution of iodine. As 1 c.c. of the thiosulphate ution should correspond to 1 c.c. of the iodine solution, the neces- y amount of water which must be added to the former is then ermined.

Method.—100 c.c. of urine, or less if much acetone is present, determined by Legal's test, are treated with 2 c.c. of the tic acid solution and distilled until seven-eighths of the total ount have passed over. The distillate is received in a retort ich is connected with a bulb tube containing water. As soon seven-eighths of the urine have distilled over, a small amount the distillate of the remainder is tested for acetone according Lieben's method. Should a positive reaction be obtained, it l be necessary either to repeat the entire process with less urine, diluted to about 200 c.c., or to add about 100 c.c. of water to the residue and to distil until all the acetone has passed over. The distillate is then treated with 1 c.c. of the sulphuric acid and redistilled. The addition of the acetic acid and of the sulphuric acid, respectively, serves the purpose of holding back phenol and ammonia. Should the first distillate contain nitrous acid, moreover, which is recognized by the addition of a little starch paste containing a trace of potassium iodide, when the solution turns blue, the acid is removed by adding a little urea. The second distillate is received in a bottle provided with a well-ground glass stopper, and holding about 1 liter. The distillate is then treated with a carefully measured quantity of the one-tenth normal solution of iodine—about 10 c.c. for 100 c.c. of urine—and sodium hydrate solution until the iodoform separates out. To this end a slight excess of the solution must be added. Should ammonia be present, a blackish cloud will be observed at the zone of contact of the sodium hydrate and the iodine solution, and it will be necessary to repeat the entire process. The bottle is closed and shaken for about one minute. The solution is then acidified with concentrated hydrochloric acid, when the mixture assumes a brown color if iodine is present in excess. If this does not occur more of the iodine solution must be added and the process repeated until an excess is present. The excess is then retitrated with the thiosulphate solution until the fluid presents a faint-yellow color. A few cubic centimeters of starch solution are now added, and the titration continued until the last trace of blue has disappeared. The number of cubic centimeters employed in the titration is finally deducted from the total amount of the iodine solution added, and the result multiplied by 0.976. The figure thus obtained indicates the amount of acetone contained in the 100 c.c. of urine, in mgrms., as 1 c.c. of the thiosulphate solution is equivalent to 1 c.c. of the iodine solution, or to 0.967 mgrm. of acetone.

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[1] See Neubauer u. Vogel, Analyse des Harns, 9th ed., p. 470.

Diacetic Acid.

The occurrence of diacetic acid in the urine must always be regarded as abnormal. Its pathological significance is identical with that of acetonuria. It is met with especially in diabetes, in various digestive diseases, and in febrile diseases. In the continued fevers of childhood it is almost constantly present. H. Baldwin noted its presence in a case of pernicious vomiting of pregnancy.

Gerhardt's Test.—To demonstrate the presence of diacetic acid a few cubic centimeters of urine are treated with a strong solution of ferric chloride drop by drop. A precipitate of phosphates is filtered off, when more of the iron solution is added to the filtrate. If a Bordeaux red color appears, this may be due to diacetic acid. To ake sure another portion of urine is boiled and similarly treated. diacetic acid is decomposed on boiling no reaction at all or only a nt reddish color should be obtained. As further proof a third rtion of urine is acidified with sulphuric acid and extracted with her. The diacetic acid is thus isolated. A positive reaction, when e ethereal extract is shaken with ferric chloride will indicate the esence of diacetic acid. The color disappears on standing for venty-four to forty-eight hours. A similar reaction is obtained with alicylic acid, antipyrine, sodium acetate, and other aromatic comounds, but the color persists for days. Sulphocyanides like diacetic cid will pass into the ethereal extract, but the color does not disapear on standing.

Arnold's Test (Modified by Lipliawski).—Two solutions are employed, iz., a 1 per cent. solution of para-amido-aceto-phenone and a 1 per ent. solution of potassium nitrite. 6 c.c. of the first solution and c.c. of the second are added to an equal volume of urine, together ith a drop of concentrated ammonia. The mixture is shaken until t assumes a brick-red color. From 10 drops to 2 c.c., according to he amount of diacetic acid present, are treated with 15 to 20 c.c. of oncentrated hydrochloric acid (sp. gr. 1.19), 3 c.c. of chloroform, nd 2 to 4 drops of an aqueous solution of ferric chloride. The tube s closed with a cork and *gently* agitated (so as to avoid emulsification), when after one-half to one minute a beautiful and very chareristic violet tinge results if diacetic acid is present. In its absence he color is yellowish or slightly reddish. The violet color persists or a long time. Bilirubin, salicylic acid, phenacetin, antipyrine, henol, and other drugs are without disturbing influence upon the eaction. Highly colored urines should first be filtered through aninal charcoal.

Allard states that both Arnold's test and that of Lipliawski give positive result also with acetone, when this is present to the extent f more than 1 per cent.

LITERATURE.—v. Jaksch, Ueber Acetonurie u. Diaceturie, loc. cit. Ibid., eit. f. Heilk., 1882, vol. iii, p. 34. Schrack, Jahrbuch f. Kinderheilk., 1889, ol. xxix, p. 411. v. Arnold, Wien. klin. Woch., 1899, p. 541.

Oxybutyric Acid.

The fact that in some cases of diabetes an excessive elimination f ammonia was observed led to the belief that there must be presnt an unknown acid; this was shown to be β-oxybutyric acid. The ccurrence of this acid in the urine of diabetic patients is of great linical interest, not only from the standpoint of diagnosis, but also f prognosis and treatment. Its presence may always be regarded s indicating a severe type of the disease, and when associated with marked acetonuria and diaceturia as indicating the possible occurrence of coma.

According to Herter, the condition of diabetic coma is preceded by a period of days, weeks, or months, in which there is a large excretion of β-oxybutyric acid (20 grams or more in twenty-four hours), and in which the nitrogen in the form of ammonia is largely increased. The same writer states that patients whose urines show or have shown a large excretion of organic acids are in danger of developing diabetic coma; but the nitrogen of ammonia may temporarily rise as high as 16 per cent., and yet coma may be delayed for more than seven months. The persistent excretion of more than 25 grams of β-oxybutyric acid indicates impending coma. Important also is the observation that while as a general rule the appearance of large amounts of organic acids is associated with the presence of much sugar, a constant relation between the two does not exist. There may thus be much sugar and little or no acid in the urine, or there may be much acid and little sugar.

Besides diabetes, the substance may be met with in scarlatina, measles, scurvy, and in starving insane patients.

The presence of oxybutyric acid may be inferred in diabetic urines if after fermentation a rotation of the plane of polarization to the left is observed. Albumin, if present, must first be removed.

Quantitative Estimation according to Darmstaedter.—This method is based on the decomposition of the β-oxybutyric acid with the formation of α-crotonic acid and the estimation of the latter. This decomposition takes place according to the equation:

$$\underset{\beta\text{-oxybutyric acid.}}{CH_3.CHOH.CH_2.COOH} = \underset{\text{Crotonic acid.}}{CH_3.CH.CH.COOH} + H_2O.$$

100 c.c. of urine are rendered feebly alkaline with sodium carbonate and evaporated on a water bath almost to dryness. With the aid of 150 to 200 c.c. of sulphuric acid (50 to 55 per cent.) the residue is transferred to a liter flask, which is closed with a doubly perforated stopper. Through the one aperture a drip-tube passes, while a bent glass tube passes through the other to a condenser. Heat is applied, at first mildly, so as to avoid foaming; then vigorously. Water is allowed to enter through the drip-tube as fast as the distillate passes over. The distillation is interrupted when from 300 to 350 c.c. have been obtained, which usually takes from two to two and one-half hours. The distillate is extracted two or three times with ether. The ether is distilled off, the residue heated for a few minutes on a sand bath to 160° C. in order to drive off any fatty acids that may be present, and then dissolved on cooling with 50 c.c. of water. The solution is filtered and the filter washed with a little water. The aqueous solution of the crotonic acid is now titrated with a decinormal sodium hydrate solution, using phenolphthalein as

indicator. 1 c.c. of the soda solution corresponds to 0.0086 ım of crotonic acid. The corresponding amount of oxybutyric d is obtained by multiplying by 1.21. Sugar does not interfere :h the process.

[f it is only desired to prove the presence of oxybutyric acid in urine, this method can also be conveniently employed. The croıic acid is obtained from the ethereal extract, and recognized by melting point, 72° C. If necessary, it can be purified by solun in water and reëxtraction with a small amount of ether and)sequent evaporation, viz., distillation of the ether.

ITERATURE.—v. Jaksch, Ueber Acetonurie u. Diaceturie, loc. cit. H. Wolpe, h. f. exper. Path. u. Pharmakol., 1886, vol. xxi, p. 131. Herter, "The Acid oxication of Diabetes in its Relation to Prognosis," Jour. of Exper. Med., 1, vol. v, p. 617. E. Darmstaedter, Zeit. f. phys. Chem., 1903, vol. xxxvii, 355.

Crotonic Acid.

As has just been shown, crotonic acid is a derivative of oxybuic acid. Its presence in the urine as such has not as yet been ablished, and it is likely that statements to the contrary are based on findings of the acid in the distillate, especially when the disation has been carried on after the addition of sulphuric acid to urine. But even in the absence of a free acid a small amount crotonic acid results from oxybutyric acid on boiling.

Lactic Acid.

Sarcolactic acid is normally absent from the urine, but is met :h in pathological conditions, and particularly in hepatic diseases, the liver is normally concerned in the decomposition of lactic d and of the lactates that have been ingested with the food. As s been pointed out, moreover, there is evidence to show that portion of the nitrogen eliminated from the body reaches the er as ammonium lactate, and is here transformed into urea. a consequence, lactic acid appears in the urine whenever, as phosphorus poisoning, acute yellow atrophy, etc., extensive struction of the hepatic parenchyma occurs, and the formation urea is consequently impaired. In such cases the elimination of tic acid is associated with an increased excretion of ammonia. The ne will occur when, owing to insufficient oxygenation of the blood, power of oxidation on the part of the liver is interfered with. e accordingly find lactic acid in the urine in the chronic anemias, cases of poisoning with carbon monoxide, in association with the rious forms of circulatory and respiratory dyspnea, in cases of epilepsy immediately after the attack, following excessive muscular exercise, as in soldiers after forced marches, etc.

In order to test for lactic acid, the urine is evaporated on a water bath to a thick syrup and extracted with 95 per cent. alcohol. This is decanted off after twenty-four hours, evaporated to a syrup, acidified with dilute sulphuric acid, and extracted with ether, so long as this presents an acid reaction. The ether is then distilled off and the residue dissolved in water. This solution is treated with a few drops of a solution of basic lead acetate, filtered, the excess of lead removed by means of hydrogen sulphide, and the filtrate evaporated to dryness on a water bath, when the lactic acid will remain behind as a slightly yellowish syrup. This is then dissolved in a little water, the solution is saturated with zinc carbonate, and boiled. Zinc lactate will separate out upon evaporation, especially if a little alcohol is added, and may be recognized by the form of its crystals, viz., small prisms. These crystals are levorotatory, soluble in alcohol (1 to 1100), and contain two molecules of water of crystallization, which is lost at 105° C., so that the loss of weight after heating to this temperature must correspond to 12.9 per cent.

LITERATURE.—O. Minkowski, "Ueber den Einfluss d. Leberextirpation auf d. Stoffwechsel," Arch. f. exper. Path. u. Pharmakol., vol. xxi, p. 41; and "Ueber Ursache d. Milchsäureausscheidung nach Leberextirpation," ibid., vol. xxxi, p. 214. G. Colosanti u. R. Moscatelli, "Ueber d. Milchsäuregehalt d. menschlichen Harns," ibid., vol. xxvii, p. 158. Jnouye and Saiki, "Lactic Acid after Epileptic Attacks," Zeit. f. physiol. Chem., 1903, vol. xxxvii, p. 203.

Oxyamygdalic Acid.

Schultzen and Riess[1] discovered an acid in the urine of patients who had died from acute yellow atrophy to which they gave the formula $C_8H_8O_4$. They regard it as oxyamygdalic acid and suppose it to be derived from tyrosin, which was also found, according to the equation:

$$C_9H_{11}NO_3 + 3O = CO_2 + NH_3 + C_3H_8O_4.$$

Very curiously it was not found in cases of phosphorus poisoning, but only in acute yellow atrophy. As in this disease there is coincidently with the rapid parenchymatous destruction much extravasation of blood, Nencki has suggested that the acid in question may possibly be derived from blood pigment, especially as Küster obtained from hematoporphyrin an acid which has the formula $C_8H_8O_5$, and which thus only differs from the product of Schultzen and Riess by a plus of one atom of oxygen.

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[1] Annalen d. Charit. Krankenhauses zu Berlin, 1869, vol. xv.

Volatile Fatty Acids.

The term *lipaciduria* is applied to the elimination of volatile tty acids in the urine. This occurs under normal conditions, but ay be much more marked in disease. With an ordinary diet the gree of lipaciduria corresponds to from 50 to 80 c.c. $\frac{1}{10}$ normal lphuric acid. In febrile conditions, according to v. Jaksch and okitansky, there is an increase, which runs parallel to the height the temperature. Rosenfeld, however, has shown that this is, rictly speaking, not correct, and that an increase is only observed those febrile states in which resorption of breaking-down albuinous material is taking place, as in cases of tonsillar abscess, ptic diphtheria, putrid bronchitis, and empyema, and in general in sociation with all suppurative processes and hemorrhages within e body. Especially high values are found during convalescence om pneumonia, during the first days following crisis. This is no ubt owing to a resorption of the exudate, and is associated with increased elimination of nitrogen. Immediately before the crisis is common to meet with very low values—20 c.c.—as compared th 100 to 240 c.c. during convalescence. These observations, as osenfeld has pointed out, may be of marked value in the diagnosis obscure accumulations of pus.

A marked decrease in the amount of fatty acids is noted in uncomicated cases of erysipelas and scarlatina (30 to 50 c.c.), in measles, phtheria, and, as I have already indicated, in pneumonia preceding tive resorption of the exudate (20 to 40 c.c.).

According to some observers, the amount of fatty acids in the ine may be regarded as an index of the degree of carbohydrate ferentation in the intestinal tract. Under normal conditions this may be e case, but in disease the question is probably more complicated.

The acids in question are formic acid, acetic acid, butyric acid, d propionic acid. They may be isolated as described in the apter on the Feces.

For their *quantitative estimation* it will suffice to distil a given lume of urine with sulphuric acid and to titrate the distillate th $\frac{1}{10}$ normal sodium hydrate solution. The results are expressed terms of the corresponding number of c.c. of $\frac{1}{10}$ normal sulphuric id. 250 c.c. of the urine, which must be fresh or preserved with loroform, are distilled with 50 c.c. of dilute sulphuric acid until 0 c.c. have passed over. The residue is diluted with 200 c.c. of ter and the distillation continued as before. In this manner e danger that some hydrochloric acid may pass over is avoided, t it is well to make sure of this by testing with silver nitrate.

The method is exact; traces of benzoic acid are included, but in n these can be neglected.

LITERATURE.—v. Jaksch, Zeit. f. klin. Med., 1886, vol. xi, p. 307; and Zeit. f. physiol. Chem., 1886, vol. x, p. 536.

Blumenthal mentions a case of catarrhal jaundice in which at a time when bile again flowed into the intestine a high degree of lipaciduria occurred, viz., up to 385.2 c.c. $\frac{n}{10}$ acid in lieu of the normal 50 to 80 c.c.

Amino-acids.

Tyrosin, leucin, and glycocoll have long been known to occur in the urine in acute yellow atrophy and phosphorus poisoning, but aside from these conditions nothing further was known of the occurrence of amino-acids under other pathological conditions (barring cystinuria). Within recent years, however, and with more exact methods it has been possible to show that bodies of this order may occur under the most diverse conditions. Phenylalanin, alanin, and arginin have been found in phosphorus poisoning, besides tyrosin, leucin, and glycocoll.[1] Glycocoll indeed, according to a recent announcement by v. Noorden, is a normal constituent of the urine and may amount to 1 per cent. of the total nitrogen output. (This is in marked contrast to the statement of Ignatowski[2] that normal human urine only contains traces of amino-acids, at best, and that even after the subcutaneous injection of 6 grams of glycocoll none is demonstrable.)

Abderhalden[3] found tyrosin in a patient dying with pneumonia, who had been suffering from arteriosclerosis, myocarditis, and diabetes. In a second case of diabetes he likewise found tyrosin and obtained a marked Millon reaction. In a third case with coma tyrosin was present also during the attack, but absent in the interim. In a case of severe hepatic cirrhosis a marked β-naphthalin sulphochloride reaction occurred, but it was impossible to isolate amino-acids in pure form. The same observer also obtained tyrosin in a case of severe icterus, referable to complete occlusion by the common duct, and in a patient who had undergone prolonged narcosis; both urines gave a marked Millon reaction.[4] Ignatowski found glycocoll constantly in the urine of 7 gouty patients; in 3 of these also other amino-acids, probably leucin and aspartic acid. In pneumonia, especially about the time of the crisis and in leukemia he likewise obtained positive results.

Voegtlin and Barker note the occurrence of a distinct Millon reaction in the urine following the injection of tuberculin for diagnostic purposes.

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[1] Wohlgemuth, Zeit. f. phys. Chem., 1905, vol. xliv. Abderhalden and Bergell, ibid., 1903, vol. xxxix; Abderhalden and L. F. Barker, ibid., 1904, vol. xlii.

[2] Zeit. f. physiol. Chem., 1904, vol. xlii, p. 400.

[3] Ibid., 1905, vol. xliv, p. 50.

[4] Ibid., vol. xlv, p. 468.

In this connection the observations of Herter and Wakeman[1] and Baldwin[2] are of special interest. Using the method of Magnus-Levy[3] of balancing the total bases against the total known acids, they found that in certain conditions, notably dilatation of the stomach, rheumatoid arthritis, and cirrhosis of the liver, there was a marked excess of bases over known acid equivalents. This leads to the nference that in the diseases mentioned there must have been present some other organic acid. Magnus-Levy had in this manner previously established the presence of such acids in starvation, in intestinal disturbances, phosphorus poisoning, acute yellow atrophy, and fever.

I append a few of Baldwin's results:

APPARENT EXCESS OF ACIDS OVER BASES.

Average of 10 normal urines	0.2943
" in diabetes mellitus	2.96
" in rheumatoid arthritis (active stage)	0.7847
" " " " "	0.5598
" " " " "	0.6983
" " " " "	0.6456
" " " " (case 16) "	0.8377

Fat.—Under strictly normal conditions the urine contains no fat, while variable amounts may be found in disease. When present in large quantities, so that it is possible to recognize it with the naked eye, the condition is termed *lipuria.* Such cases, however, are rare, and the diagnosis should only be made when it is possible to exclude accidental contamination. Smaller quantities, recognizable only with the microscope, are much more common, and are indeed quite constantly observed whenever fatty degeneration of the renal epithelial cells, of pus corpuscles, or of tumor particles is taking place in the urinary tract. The fat droplets may then be found floating in the urine or attached to or embedded in any morphological elements that may be present. Lipuria may also occur when abnormally large quantities of fat are circulating in the blood. It is thus observed after the administration of cod-liver oil in large quantities, following oil inunctions, in cases of fracture of the long bones with extensive destruction of the bone-marrow, in cases of eclampsia, as also in such diseases as diabetes mellitus, chronic alcoholism, phthisis, obesity, leukemia, in certain mental diseases, in affections of the pancreas and heart, etc.

The term *chyluria* or *galacturia* has been applied to a condition in which a turbid urine presenting the macroscopic appearance of milk is excreted. Upon microscopic examination it may be demonstrated that the turbidity in such cases is owing to the presence

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[1] Trans. Assoc. Amer. Phys., vol. xv.
[2] Amer. Jour., December, 1904, p. 1038.
[3] Arch. f. exp. Pathol. and Pharmak., 1899, vol. xlii, p. 149, and ibid., 1900-1901, vol. xlv, p. 388.

of innumerable highly refractive globules of fat, which may be removed by shaking with ether. Of morphological constituents, leukocytes are occasionally encountered in large numbers. Red blood corpuscles are also seen at times, and when present in large numbers impart a rose color to the urine. Fibrinous coagula are often observed when the urine has stood for some time, and the entire bulk of urine may even become transformed into a gelatinous mass. Albumin is present in most cases in the absence of other constituents pointing to renal disease, such as tube casts and renal epithelial cells. Leucin, tyrosin, and cholesterin may also at times be found, particularly the latter. It has been quite generally accepted that chyluria is due to the presence of the Filaria sanguinis hominis; but while filarias are undoubtedly present in the blood in the majority of instances, and may also be present in the urine, it has been demonstrated that cases occur in which filariasis does not exist.

LITERATURE.—Lipuria: Schütz, Prag. med. Woch., 1882, vol. vii, p. 322. Ebstein, Arch. f. klin. Med., 1879, p. 115. Chyluria: Huber, Virchow's Archiv, 1886, vol. cvi, p. 126. Rossbach-Götze, Verhandl. d. Congr. f. inn. Med., 1887, vol. vi, p. 212. Brieger, Zeit. f. physiol. Chem., 1880, vol. iv, p. 407. Grim, Langenbeck's Archiv, 1885, vol. xxxii, p. 511.

Ferments.

Ferments may be demonstrated in every urine, both under physiological and pathological conditions. Pepsin is said to be absent in cases of typhoid fever, carcinoma of the stomach, and possibly also in nephritis. In order to demonstrate its presence, a small flake of boiled fibrin is placed in the urine, and after several hours removed to a 2 to 3 pro mille solution of hydrochloric acid. The pepsin, if present, will be deposited upon the fibrin and effect digestion of the latter in the hydrochloric acid solution.

Diastase, a milk-curdling ferment, and a fat-splitting ferment have also been observed. It is noteworthy that the fat-splitting ferment was first encountered in a case of hemorrhagic pancreatitis, and it has been suggested that its presence may possibly be of value in the diagnosis of the disease. Opie, who reports the case, demonstrated its presence by the method of Kastle and Loevenhart. Only a small amount of urine was obtained. This was neutralized with $\frac{n}{10}$ alkali and divided into two portions. To one portion were added 0.25 c.c. of ethyl butyrate together with a small quantity of litmus solution and 0.1 c.c. of toluol. The second portion used as a control was boiled in order to destroy the ferment if present, and ethyl butyrate added. Both specimens were kept at 37° C.; at the end of twenty-four hours the unboiled specimen had acquired a well-marked acid reaction, while the control specimen was little if at all changed. A quantitative estimation can be made by titrating the two specimens ith $\frac{n}{10}$ alkali (using phenolphthalein as an indicator), and noting he amount of ethyl butyrate which is split by the ferment. The itration should be made after adding to each specimen 0.5 c.c. more f $\frac{n}{10}$ HCl than of the $\frac{n}{10}$ alkali originally used, and to shake out the utyric acid with 50 c.c. of ether and 25 c.c. of alcohol; the acid is then itrated directly in the ethereal solution.

Since the diagnosis of acute lesions of the pancreas is difficult and t times impossible the demonstration of the constant occurrence of the ferment under such circumstances would be of great importance. Its diagnostic importance has been further emphasized by the experimental work of Hewlett on dogs (which see).

LITERATURE.—Opie, Johns Hopkins Hospital Bull., 1902, vol. xiii, p. 117. Kastle and Loevenhart, Amer. Chem. Jour., vol. xxiv. Hewlett, Jour. of Med. Research, May, 1904, p. 377. Garnier, Compt.-rend., 1903, vol. v, p. 1064.

Gases.

Every urine contains a small amount of gases, notably carbon dioxide, oxygen, and nitrogen, which may be withdrawn by means of of an air-pump.

Under pathological conditions hydrogen sulphide is at times also found, constituting the condition known as *hydrothionuria.* In some instances this is referable to a diffusion of the gas into the bladder from neighboring organs or accumulations of pus; but this is rare. In others an abscess has ruptured into the bladder, or a direct communication exists between it and the bowel. Under such conditions it can, of course, not be surprising that hydrogen sulphide together with other products of albuminous putrefaction are eliminated in the urine. More commonly, however, the hydrothionuria occurs idiopathically, and is then referable to the action of certain microörganisms. This can be readily demonstrated by adding a few cubic centimeters of such urine to normal urine, when upon standing the formation of hydrogen sulphide may be demonstrated in the normal specimen. The common organisms, however, which cause ammoniacal decomposition apparently have no part in this process, and the formation of the hydrogen sulphide may be observed before ammoniacal decomposition has set in, and while the reaction is yet acid. If a small amount of ordinary decomposing urine, moreover, is added to fresh normal urine, no hydrogen sulphide is, as a rule, produced. The character of the organisms in question is variable; sometimes micrococci are found, at other times bacilli, and in still other instances both. Besides being capable of producing hydrogen sulphide from the sulphur bodies of the urine, some of them also cause the formation of ammonium carbonate in dilute solutions of urea.

The source of the hydrogen sulphide in cases of hydrothionuria is in most cases probably the so-called neutral sulphur, but it is possible that the oxidized sulphur is at times also attacked. In cystinuria, in which the neutral sulphur is more or less increased, hydrothionuria is commonly observed. Its occurrence in such cases is indeed so frequent that I am inclined to suspect cystinuria, although crystals of cystin are not found in the sediment.

In a few recorded instances the hydrothionuria accompanied indigosuria, viz., the presence of free indigo blue in the urine; and this Müller has likewise shown to be referable to the action of certain microörganisms. (See Indigosuria.)

The formation of hydrogen sulphide in decomposing urines containing albumin is, of course, common, and should not be confused with the idiopathic hydrothionuria here described.

The chemical test for hydrogen sulphide is very simple: a strip of filter paper is moistened with a few drops of sodium hydrate and lead acetate solution and clamped into the neck of the bottle containing the urine. After a variable length of time, in some instances immediately, in others only after twelve to twenty-four hours, a discoloration of the paper will be observed, varying from a grayish brown to black according to the amount present. When this is large it is, of course, also recognized by its characteristic odor.

LITERATURE.—F. Müller, "Schwefelwasserstoff im Harn," Berlin. klin. Woch., 1887, Nos. 23 and 24. Rosenheim u. Gutzmann, Deutsch. med. Woch., 1888, No. 10. Kahler, Prag. med. Woch., 1888, No. 50.

Ptomains.

Numerous researches have shown that traces of toxic alkaloidal substances may be encountered in the urine under the most diverse pathological conditions, and may be present even in health. Of the nature of these bodies, however, little is known. Thudichum claims to have isolated three distinct basic substances from normal urine, which he has termed *reducin*, *parareducin*, and *aromin*. Pouchet and Mme. Eliacbeff, working in Gautier's laboratory, have likewise extracted toxic bodies from normal urines; and Adduco states that after fatiguing exercise, especially, he could demonstrate in the urine a substance which was extremely toxic, and was not identical with cholin, as was first supposed. All this work, however, must be repeated with great care before the results obtained can be regarded as conclusive. This is also true of the work which has been done in various diseases. Some observers have here described bodies which they regard as specific toxins. Griffith thus reports the presence of a specific poison of scarlatina, of measles, mumps, etc. His results, however, do not invite confidence and have never been confirmed either by himself or by others.

The only substances belonging to the class of ptomains which have thus far been obtained from the urine in amounts sufficient to establish their identity are *cadaverin* and *putrescin*. They were originally discovered by Brieger in putrefying cadavers, and subsequently also found in cultures of the bacillus of Asiatic cholera, the Finkler-Prior bacillus of cholerina, the bacillus of tetanus, and in the rice-water stools of cholera patients. From the urine cadaverin, putrescin, and a third diamin isomeric with cadaverin, which has been regarded as saprin or neuridin, were first obtained by Baumann and v. Udranszky in a case of cystinuria, and it appears that diaminuria occurs only in association with this disease. All attempts to isolate diamins from the urine under other pathological conditions at least have given rise to negative results. Regarding the origin of the ptomains in question there can be no doubt, I think, that they are derived from the corresponding hexon bases, arginin and lysin, as the result of a definite metabolic anomaly, of which the cystinuria is also one expression. I have advocated this view for some years, and Löwy and Neuberg have recently furnished the experimental proof for this supposition. They found in a cystinuric individual who was not excreting any diamins that putrescin and cadaverin appeared when the corresponding hexon bases were ingested. Löwy and Neuberg further claim to have found tyrosin and aspartic acid when these were given by the mouth, which would tend to show that in the cystinuric there is even a more extensive inability to oxidize amino-acids than the cystinuria and diaminuria alone would indicate. I have not been able to verify these findings, however, so far as tyrosin is concerned, and Folin also obtained negative results.

Putrescin has been found by Baumann and v. Udranszky, Bödtker, and Garrod. Brieger, Stadthagen, Leo, Garrod, Lewis, and I have succeeded in isolating cadaverin from such urines. Others have been less successful. As regards the question whether diaminuria and cystinuria invariably coexist I have shown that this is not always so, and that the two conditions may alternate, and that the one may temporarily disappear while the other continues. Whether or not cases occur in which diamins are constantly absent I am not prepared to say. Cases have been reported by Garrod and others in which no diamins could be found, but it is possible that our analytical methods are not sufficiently delicate to demonstrate mere traces.

The amount of diamins which may be met with in the urine of cystinuric patients is extremely variable. In one case I was able to isolate 1.6 grams of the benzoylated cadaverin from the collected urine of twenty-four hours. On other days traces only were present, and at times no diamins at all could be found. In the case of Dr. Lewis, I obtained only 0.3 gram from 12,000 c.c.

Isolation of Diamins. Method of Baumann and v. Udranszky.—The collected urine of at least twenty-four hours is shaken with a

10 per cent. solution of sodium hydrate and benzoyl chloride in the proportion of 1500 to 200 to 25 until the odor of the benzoyl chloride has entirely disappeared. The resulting precipitate contains phosphates, the benzoyl compounds of the normal carbohydrates of the urine, and a portion of the benzoylated diamins. These are filtered off with the aid of a suction pump and digested with alcohol. The filtered alcoholic extract is concentrated to a small volume and poured into about 30 times its amount of water. Upon standing for from twelve to forty-eight hours the benzoylated diamins separate out in the milky fluid in the form of a more or less voluminous sediment composed of fine, intensely white crystals. In order to remove the benzoylated carbohydrates likewise present, the precipitate is redissolved in alcohol, the solution concentrated to a small volume, and diluted with water as described. This process is repeated several times. The resulting crystals, if both diamins are present, will lose their water of crystallization at 120° C. and melt at 140° C.

A smaller portion of the benzoylated diamins remains in the first filtrate. In order to recover this the filtrate is acidified with sulphuric acid and extracted with ether. The ethereal residue, before congealing, is placed in as much of a 12 per cent. solution of sodium hydrate as is required for its neutralization, when from 3 to 4 times the volume of the same solution is added. This mixture is placed in the cold, when long needles and platelets separate out, which consist of the sodium compound of benzoyl cystin and the benzoylated diamins. The sediment is filtered off and placed in cold water, in which the sodium-benzoyl cystin dissolves, while the benzoylated diamins remain undissolved.

In order to separate the putrescin from the cadaverin, the crystals are dissolved in a little warm alcohol and treated with 20 times the volume of ether. Benzoyl putrescin is thus thrown down, and may be recognized by its melting point, viz., 175° to 176° C., while the ethereal residue contains the benzoyl cadaverin, which melts at from 129° to 130° C.

The diamins may then be separated from the benzoyl radicle by heating the crystals on a water bath with a mixture of equal parts of alcohol and concentrated hydrochloric acid until a specimen is entirely dissolved by sodium hydrate. The separation is complete after from twenty-four to forty-eight hours, according to the amount present. The solution is then diluted with water, when the benzoic acid, which has been formed, separates out and is filtered off. After extracting with ether, in order to remove any benzoic acid still remaining, the filtrate is evaporated to dryness. A crystalline mass remains, which is easily soluble in water, but with difficulty in alcohol. This consists of putrescin and cadaverin hydrochlorates, from which the various double salts with platinum, silver, mercury, etc., can be readily obtained. The platinum salt of cadaverin is formed by adding an alcoholic solution of platinum chloride to a solution of the hydrochlorate in alcohol; it occurs as a voluminous yellow, crystalline mass, which can be purified by recrystallization from hot water. When this salt is decomposed by hydrogen sulphide the hydrochlorate again results, from which the free base is obtained by distillation with caustic potash. During this distillation water passes over at first; and above 160° C. a colorless oil appears, the boiling point of which is about 173° C. This constitutes the free base, which may be identified by its sperm-like odor and the avidity with which it attracts carbon dioxide from the air to form carbonate.

Phenylcyanate Method (Löwy and Neuberg).—This method has certain advantages over the one preceding and may be utilized in doubtful cases. In aqueous solution phenylcyanate does not unite with carbohydrates and the cystin derivative does not separate out in the presence of free alkali, but only upon the addition of an acid.

The urine is feebly acidified with sulphuric acid and precipitated with phosphotungstic acid. The precipitate is collected on a filter, washed with 5 per cent. sulphuric acid, suspended in water, and the adherent sulphuric acid counteracted with a little baryta. The solution is then heated to 50° C. and treated with a concentrated solution of barium hydrate, also heated to 50° C. until a slight excess is demonstrable in the filtrate by means of sodium carbonate. The precipitate is removed by filtration and the excess of barium by means of a current of carbon dioxide. The resultant solution is treated with normal alkali (about 40 c.c.), and phenylisocyanate now added drop by drop. During this process there is a distinct evolution of heat. A voluminous precipitate is formed, which is filtered off. This is almost insoluble even in boiling alcohol, but dissolves in pyridin. From the resultant solution the phenylcyanate of the diamins separates out upon the careful addition of water, in snowy white crystals. They can be purified by repeated solution and reprecipitation. The putrescin compound can be separated from the cadaverin derivative by adding water-free acetone to the concentrated solution in pyridin, when the putrescin phenylcyanate is thrown down at once, while the other only separates from the filtrate after standing for several hours. The melting point of the cadaverin compound is 207° C. and of the putrescin derivative 238° to 240° C.

LITERATURE.—Stadthagen, "Ueber d. Harngift," Zeit. f. klin. Med., 1889, vol. xv, p. 383. Bouchard, Compt.-rend. Soc. de biol., 1884; and Compt.-rend. de l'Acad. des sci., vol. cii, p. 1127. Lépine et Aubert, ibid., vol. ci, p. 90. Adduco, Arch, ital. d. Biol., vol. ix, p. 203, and x, p. 1.

Diaminuria: v. Udranszky u. Baumann, Zeit. f. physiol. Chem., 1889, vol. xiii, p. 562. Stadthagen u. Brieger, Berlin. klin. Woch., 1889, vol. xxvi, p. 344. Bödtker, Norsk. Mag. f. Laegevidensk., 1892, vol. vii, p. 1220. Moreigne, Arch. de Méd. exper. et d'Anat. path., 1899, p. 254. Simon, Amer. Jour. Med. Sci., 1900, vol. cxix, p. 39. Garrod and Cammidge, Jour. Path. and Bact., Feb., 1900. Bödtker, Zeit. f. phys. Chem., 1905, vol. xlv, p. 393. Löwy and Neuberg, Zeit. f. phys. Chem., 1904, vol. xliii, p. 338.

KRYOSCOPIC EXAMINATION OF THE URINE.

The kryoscopic examination of the mixed urine does not furnish as valuable information as the corresponding examination of the blood. This is largely owing to the fact that the normal variations in the freezing point of the urine are much more extensive—*i. e.*, between —0.9° and —2° C. In the determination of renal insufficiency, however, where specimens from each kidney separately are available, or at least one specimen from one kidney together with a mixed specimen from the same patient, the method furnishes very satisfactory results; it indicates the location of the disease more definitely than a quantitative estimation of urea, tests of specific gravity, and the other usual tests of the urine. Especially interesting are the results which are obtained in cases of unilateral disease of the kidneys in which the other organ is functioning normally; kryoscopic examination of the blood will then furnish normal values as there is normal elimination, while a separate examination of the urine from the two sides reveals the diseased kidney. A value of Δ higher than —0.9° C. is abnormal.

In pneumonia, Schmidt found the freezing point much lowered. It does not rise to normal until after that of the blood, *i. e.*, several days after the crisis.

The examination is conducted as described in the case of the blood.

Literature.—See Kryoscopy of the Blood.

MICROSCOPIC EXAMINATION OF THE URINE.

Sediments.

In the chapter treating of the general physical characteristics of the urine it was stated that, on standing, every urine gradually becomes cloudy owing to the development of the so-called nubecula. This was shown to consist of a few mucous corpuscles, a small number of pavement epithelial cells derived from the urinary and genital passages, and under certain conditions of a few crystals of uric acid, of calcium oxalate, or of both. It was further pointed out that owing to a diminution in the acidity of the urine on standing, in consequence of an interaction between the neutral sodium urate and the acid sodium phosphate, a sediment is thrown down which consists of acid sodium urate, and at times of free uric acid (see Reaction). Should the reaction of the urine be alkaline, however, when freshly voided, a condition which may occur physiologically, when t is dependent upon the ingestion of large quantities of vegetables ich in organic salts of the alkalies, but which may also be due to mmoniacal decomposition, those constituents of the urine which are held in solution merely in consequence of the presence of acid sodium phosphate are also thrown down. In that case the sediment consists essentially of calcium, magnesium, and ammonium salts. Crystals of ammonio-magnesium phosphate, it is true, may also be observed in alkaline urines of the first variety, but they are then almost always due to an increased elimination of ammonia, and hence are rarely observed under physiological conditions.

Normally calcium is found only in combination with phosphoric acid and carbonic acid. Of the three possible calcium salts of phosphoric acid—*i. e.*, $Ca_3(PO_4)_2$, $CaHPO_4$, and $Ca(H_2PO_4)_2$—only the first two are found in an alkaline urine, but they may also be observed in specimens which are either neutral or but faintly acid. The acid calcium phosphate, $Ca(H_2PO_4)_2$, is seen but rarely in sediments; it is precipitated together with uric acid and under similar conditions. Calcium carbonate, $CaCO_3$, is seen only in neutral or alkaline urines. As soon as ammoniacal fermentation has begun, ammonium salts are formed, viz., ammonium urate and ammonio-magnesium phosphate.

The following table shows the various mineral constituents usually observed in sediments, the reaction of the urine being in every case the all-important factor:

Reaction acid:
  Uric acid.
  Sodium urate.
  Calcium oxalate.
  Primary calcium phosphate.
  Ammonio-magnesium phosphate.
Reaction alkaline (referable to fixed alkalies):
  Secondary calcium phosphate.
  Tricalcium phosphate.
  Calcium carbonate.
  Ammonio-magnesium phosphate.
Reaction alkaline (referable to ammonia):
  Ammonium urate.
  Ammonio-magnesium phosphate.
  Tricalcium phosphate.
  Calcium carbonate.

In pathological conditions still other unorganized substances may be observed, viz., cystin, xanthin, hippuric acid, indigo, urorubin, hematoidin, magnesium phosphate, calcium sulphate, cholesterin, leucin, tyrosin, fats, soaps of magnesium and calcium, etc. Of these, cystin, xanthin, hippuric acid, tyrosin, calcium sulphate, hematoidin, magnesium phosphate, leucin, and the soaps of magnesium and calcium occur principally in acid urines, while indigo, urorubin, and cholesterin are usually only found in alkaline specimens. Before considering these various constituents in detail, a few words regarding sediments in general and the method to be followed in their microscopic examination may not be out of place.

An idea of the nature of a deposit may often be formed by simple inspection, especially if the reaction of the urine is known.

A crystalline sediment, presenting a brick-red color and appearing to the naked eye like cayenne pepper, is referable to uric acid. On the other hand, a salmon-red, amorphous deposit occurring in an acid urine consists essentially of sodium urate. Should doubt be felt, it will only be necessary to heat the urine, when the urate deposit will dissolve. A white, flocculent sediment in an alkaline urine is usually referable to a mixture of phosphates and carbonates, and will dissolve upon the addition of acetic acid, but remains unaffected by heat.

A sediment consisting of pus, and occurring in alkaline urines, is frequently mistaken for a phosphatic deposit by the beginner. Aside from a microscopic examination, the question may be settled by the addition of a small piece of caustic soda and stirring, when in the presence of pus the liquid becomes mucilaginous and ropy. If much pus is present, a tough, jelly-like mass will be formed, which escapes from the vessel *en masse* when the urine is poured out. Such a sediment, moreover, does not disappear upon the addition of an acid, and is rendered still more dense upon the application of heat.

Blood when present beyond traces may also be recognized.

As a general rule, the non-organized elements of a sediment are of little clinical interest.

Students are frequently in the habit of diagnosticating an excessive, normal, or subnormal elimination of one or another urinary constituent from the result of a microscopic examination. This is unwarrantable. It should always be remembered that no conclusions whatsoever can be drawn in this manner as to the amount actually eliminated. Nothing would be more erroneous than to infer an excessive excretion, not to speak of an excessive production of uric acid or of oxalic acid from the fact that crystals of these substances are seen in large numbers under the microscope. Again and again cases are observed in which an excessive elimination of uric acid, oxalic acid, or phosphates is thus diagnosticated in which chemical analysis shows not only no increase but even a diminution of the normal quantity.

A urine which is turbid when passed may be examined microscopically at once. As a rule, however, it is necessary to wait until a sediment has formed. To this end the urine should be kept in a clean and well-stoppered bottle. A small amount of chloroform is added if necessary, and will preserve the specimen almost indefinitely. A few drops of the sediment are then removed by means of a *clean* pipette, carried down to the sediment, with the distal end tightly closed by the finger, care being taken not to allow the urine to *rush* into the tube by suddenly releasing the pressure, but withdrawing an amount just sufficient for an examination. This is then spread over a *clean slide* that has been moistened by the breath, when the specimen may be examined at once. *Covering the specimen with a slip is unnecessary. A low power of the microscope* (B. & L. ⅔; Leitz 3) *should always be employed, and the high power only used to study details of structure.*

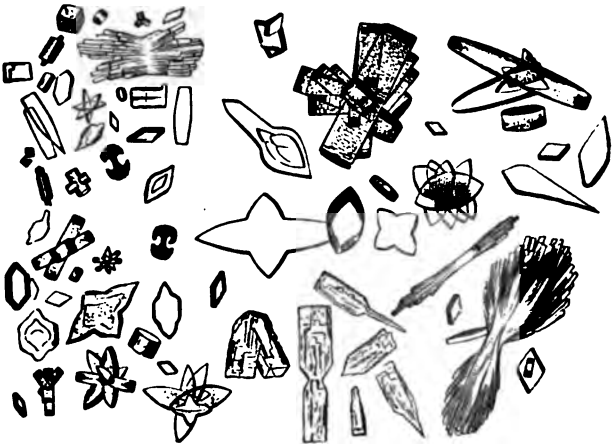

FIG. 151.—Various forms of uric acid crystals. (Finlayson.)

If a centrifugal machine is available, it is, of course, not necessary to let the urine stand until a sediment has formed. An amount sufficient for a microscopic examination can then be obtained in a few minutes.

Non-organized Sediments.

Sediments Occurring in Acid Urines. Uric Acid.—The form which uric acid crystals may present in a deposit varies greatly, the most common being the so-called whetstone form (Figs. 143 and 151). The crystals may occur singly or in groups. Accidental impurities, such as threads or hairs, are at times covered with such crystals, forming long cylinders. Very frequently uric acid crystallizes in the form of large rosettes of drawn-out whetstone crystals, presenting a brownish-red color, referable to uroerythrin, when they are often visible to the naked eye, and form the well-known *brick-dust sediment.* While it is generally stated that uric acid crystals can always be recognized by their color, which may vary from a light yellow to a dark brown, this is, in my experience, not the case. I have often seen uric acid sediments in which the crystals formed small rhombic plates with rounded edges, and were absolutely devoid of coloring matter, so far as a microscopic examination could show. Uric acid "dumb-bells" are also at times observed, and may be mistaken for calcium oxalate. Hexagonal plates of uric acid have been similarly confounded with cystin.

A uric acid sediment may be observed in cases in which an increased excretion of uric acid occurs, but it does not follow that a uric acid sediment indicates an increased elimination. Such an assumption would only be warrantable if a quantitative estimation had been made. It is more common to meet with uric acid sediments where the actual amount is not increased than the contrary. Brick-dust sediments are frequently observed during cold weather, but it would be erroneous to infer an increased elimination from such an occurrence, as the phenomenon is owing to the fact that uric acid is less soluble in cold than in warm water. During the summer months, for the same reason, a deposit of uric acid is less frequently observed, although an increased amount may nevertheless be present, being held in solution owing to the higher temperature. The more concentrated the urine, the more readily will such a deposit form. It is hence noted after profuse perspiration, following severe muscular exercise, in acute rheumatism with copious diaphoresis, in acute gastritis and enteritis associated with copious vomiting or diarrhea, during the crisis of pneumonia (particularly if accompanied by much sweating), etc.

Where a distinct tendency exists for the separation of uric acid sediments, as in cases where the urine is habitually over-acid, the possibility of the same occurrence within the urinary passages should be borne in mind (gravel, calculus). F. Müller has recently shown that the habitual separation of uric acid from the urine which is noted in such cases and which is commonly associated with vague dyspeptic and nervous disturbances is referable to the presence in such urines of considerable amounts of organic acids of unknown composition.

Chemically, the nature of a uric acid sediment may be recognized by the fact that the crystals dissolve upon the addition of sodium hydrate and reappear in the rhombic form upon acidifying with hydrochloric acid. When heated with dilute nitric acid the beautiful red color of ammonium purpurate is obtained upon the subsequent addition of ammonia (murexid test), as described elsewhere.

Amorphous Urates.—Sodium and potassium urates frequently, and especially in fevers, form sediments of such density that upon microscopic examination it is almost impossible to discern anything but innumerable amorphous granules scattered over the entire field and obscuring all other elements that may be present. Cells or casts will frequently be seen studded with these granules. In such cases it is best to heat the urine to a temperature of 50° C., and to centrifugalize the urine as soon as it has cleared.

Urate sediments are always colored, the tint varying from a dirty yellowish brown to a bright salmon red, owing to the presence of uroerythrin. Difficulties can hence never arise in determining the nature of the sediment, as a colored deposit appearing in an acid urine which dissolves upon the application of heat cannot be due to anything but urates. If a drop of the sediment, moreover, is treated upon a slide with a drop of hydrochloric acid, characteristic whetstone crystals of uric acid separate out, but the greater portion appears in the form of rhombic platelets.

Calcium Oxalate.—This substance generally appears in urinary sediments in the form of colorless, highly refractive octahedra (Fig. 152), which vary greatly in size; some appear as mere specks under even a comparatively high power, while others may attain the dimensions of a large leukocyte. Frequently one axis is longer than the other. From the fact that their diagonal planes are highly refractive, apparently dividing the superficial plane into four triangles, they have been compared to envelopes, and it is this envelope form of the crystals which is especially characteristic. In the same specimen of urine so-called dumb-bell forms may be seen, which appear to be made up of two bundles of needle-like crystals united in the form of the figure 8. Other forms may also be seen, and are shown in the accompanying figure.

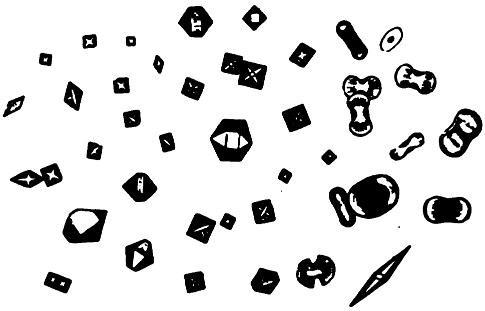

FIG. 152.—Calcium oxalate crystals. (Finlayson.)

While the envelope crystals are highly characteristic and can hardly be mistaken for any other substance, the student may at times confound them with crystals of ammonio-magnesium phosphate. This error may be avoided if it is remembered that the calcium oxalate crystals are usually not so large as those of the magnesium salt, and that the latter dissolve upon the addition of acetic acid, in which calcium oxalate is insoluble. The distinction from uric acid, if we are dealing with the dumb-bell form, cannot always be made by mere inspection. A drop of caustic soda should be added, which will dissolve the crystals if they are uric acid, while calcium oxalate remains unchanged.

It has been pointed out that under strictly normal conditions a few isolated crystals of calcium oxalate may be found in the primitive nubecula, so that their presence in urinary sediments cannot be regarded as pathological. After the ingestion of certain vegetables and fruits, notably tomatoes, rhubarb, garlic, asparagus, and oranges, or following the continued administration of sodium bicarbonate or the salts of vegetable acids, calcium oxalate crystals may be observed in large numbers; so also in certain diseases, such as diabetes mellitus, catarrhal jaundice, phthisis, emphysema, etc.

As in the case of uric acid, no inference as to the quantity eliminated can be drawn from a microscopic examination of the sediment. The frequent occurrence of abundant sediments of this substance may, however, generally be regarded as abnormal, providing that such an occurrence cannot be explained by the nature of the diet. It is very suggestive to note the frequency with which such sediments are observed in cases of neurasthenia associated with a mild degree of albuminuria, as also in various digestive neuroses. Finally, as with uric acid, the possibility of the formation of renal calculi should be borne in mind whenever abundant sediments of calcium oxalate are encountered upon frequent examination.

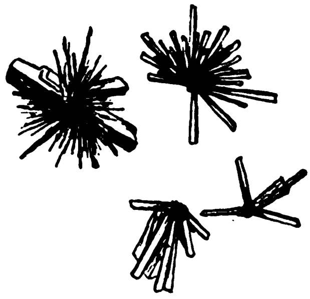

Fig. 153.—Monocalcium phosphate crystals.

Monocalcium phosphate crystals are rarely seen, and only in specimens presenting a highly acid reaction, when uric acid crystals are also frequently observed in large numbers (Fig. 153). They are colorless and soluble in acetic acid.

Hippuric acid crystals have been observed, although rarely, in urinary sediments, in acute febrile diseases, diabetes, and chorea, while their occurrence following the ingestion of large amounts of prunes, mulberries, blueberries, or the administration of benzoic acid and salicylic acid, is more common.

Hippuric acid occurs in the form of fine needles or rhombic prisms and columns, the ends of which terminate in two or four planes, at times resembling the crystals of ammonio-magnesium phosphate and of uric acid. From the former they may be readily distinguished by their insolubility in hydrochloric acid, and from the latter by the fact that they do not give the murexid reaction when treated with nitric acid and ammonia. In the case of urines rich in hippuric acid in which the substance does not appear in the sediment, it is well to add a small amount of hydrochloric acid, when the crystals will gradually separate out. Their presence does not appear to possess any clinical significance.

Calcium sulphate, in the form of long, colorless needles or elongated prismatic tablets (Fig. 154), has been observed in urinary sediments in only two cases. In both the urine, especially on standing, deposited a milky-looking sediment, the reaction being strongly acid. It may be recognized by its insolubility in acids and ammonia.[1]

Cystin is rarely seen in urinary sediments. It occurs in the form of colorless, hexagonal platelets, which are very characteristic (Fig. 155). The crystals are soluble in ammonia and hydrochloric acid, and insoluble in acetic acid, water, alcohol, and ether. They can thus be readily distinguished from certain forms of uric acid, with which they might possibly be confounded at first sight. When heated upon platinum foil they burn with a bluish-green flame without melting.

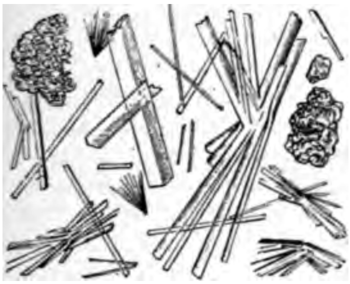

FIG. 154.—Calcium sulphate crystals. (v. Jaksch.)

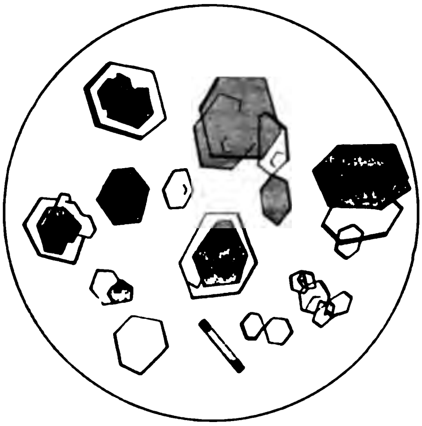

FIG. 155.—Crystals of cystin spontaneously voided with urine. (Roberts.)

Cystin-containing urines may be of normal appearance, but they often present a peculiar greenish-yellow color. The reaction is mostly neutral or alkaline. Upon standing exposed to the air a marked odor of hydrogen sulphide develops, owing to decomposition of the cystin; but at times urines are met with in which a distinct odor of hydrogen sulphide is noticeable, although crystals of cystin are not seen in the sediment. It may then be demonstrated by strongly acidifying the urine with acetic acid or by allowing it to undergo ammoniacal decompostion. In either case cystin crystals will separate out on standing. It should be remembered, however, that not all urines in which hydrogen sulphide is formed contain cystin. (See Hydrothionuria.)

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[1] v. Jaksch, Zeit. f. klin. Med., 1892, vol. xxii, p. 554.

The amount of cystin which may be found in urinary sediments is variable. Sometimes a few crystals only are obtained, while at others from 0.5 to 1 gram may be recovered. As is the case with the other non-organized constituents of sediments, however, the amount deposited does not necessarily indicate the total amount present. Where a quantitative estimation of cystin is to be made, it is best to filter off that which is deposited and to estimate the amount of neutral sulphur in the filtered urine. An increase beyond the normal may be referred to the cystin remaining in solution. (See Neutral Sulphur.)

Clinical interest in connection with cystinuria centres in the frequent association of cystin sediments with cystin gravel or calculi; but the cystinuria may exist for years without giving rise to clinical symptoms.

Very remarkable is the not uncommon occurrence of cystinuria in families. Cases of transient cystinuria likewise occur, and it is hence scarcely admissible to speak of a "cured" cystinuria when the condition disappears under some supposed treatment.

Of the origin of the condition little is known. It has been supposed that the appearance of cystin in the urine is in some manner connected with the formation of certain diamins in the intestinal canal. I have pointed out, however, that in all probability the formation of cystin and diamins takes place in the tissues of the body, and that the appearance of both is the expression of a definite metabolic anomaly rather than of a specific infection. (See Ptomains and Neutral Sulphur.)

LITERATURE.—C. E. Simon, "Cystinuria and its Relation to Diaminuria," Amer. Jour. Med. Sci., 1900, vol. cxix, p. 39. See also the literature under Ptomains.

Leucin and **tyrosin** are never found in urinary sediments under normal conditions. They are seen especially in acute yellow atrophy and in some cases of acute phosphorus poisoning.

Traces of leucin and tyrosin are said to be constantly present also in cases of cirrhosis and carcinoma of the liver, in cholelithiasis, catarrhal jaundice, Weil's disease, nephritis, cystitis, gout, bronchitis, tuberculosis, typhoid fever, hysteria, erysipelas, glucosuria, etc., but in many cases the proof has not been properly furnished that the substance under examination was really tyrosin. In connection with cystinuria the elimination of tyrosin has also been observed, but in two cases which I examined in this direction I obtained negative results.

Isolation of Leucin and Tyrosin.—As leucin is hardly ever found in the sediment, and tyrosin only when present in large quantities, the urine in every case should first be concentrated upon a water bath and examined on cooling. At times, however, when these substances are present in only very small quantities, this procedure may not lead to the desired end, and in doubtful cases the following method should be employed:

The total amount of urine voided in twenty-four hours is precipitated with basic lead acetate and filtered, when the filtrate, from which the excess of lead has been removed by means of hydrogen sulphide, is evaporated to as small a volume as possible. Any urea that may be present is removed by shaking with a small amount of absolute alcohol, and the insoluble residue extracted with alcohol containing a little ammonia. This extract is concentrated to a small volume and left to spontaneous crystallization. Leucin and tyrosin separate out and can then be further examined.

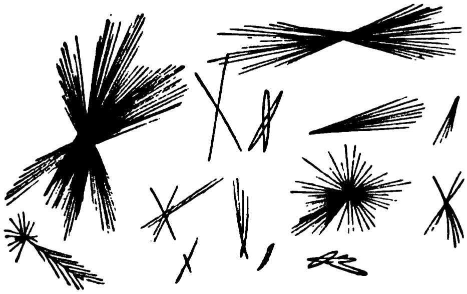

Fig. 156.—Tyrosin crystals. (Charles.)

Ulrich advises to evaporate the urine to dryness and to heat the residue gently while the vessel is covered with a plate of glass or a funnel. The tyrosin is then said to sublime, and is deposited on the cool glass in crystalline form, the crystals giving the characteristic reactions.

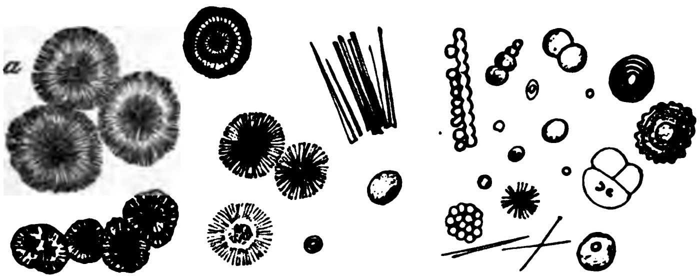

Fig. 157.—Crystals of leucin (different forms). (Crystals of kreatinin-zinc chloride resemble the leucin crystals depicted at *a*.) The crystals figured to the right consist of comparatively impure leucin. (Charles.)

Tyrosin crystallizes in the form of very fine needles (Fig. 156), which are usually grouped in sheaves. They are insoluble in acetic acid, but soluble in ammonia and hydrochloric acid.

Leucin (Fig. 157) occurs in the form of spherules of variable size, which resemble globules of fat, but may be distinguished from these by their insolubility in ether. In the urine they present a more or less pronounced brownish color, and upon close examination concentric striations as well as very fine radiating lines can at times be made out, which are especially characteristic.

If crystals resembling tyrosin and leucin are found, the following tests should be made:

Separation of the Tyrosin.—The crystals are filtered off, washed with water, and dissolved in ammonia to which a little ammonium carbonate has been added. The solution is allowed to evaporate, when the tyrosin separates out.

Piria's Test.[1]—A bit of the tyrosin is moistened on a watch-crystal with a few drops of concentrated sulphuric acid, covered, and set aside for half an hour. It is then diluted with water, heated, and while hot saturated with calcium carbonate and the solution filtered. The filtrate is colorless, but when heated with a few drops of a very dilute neutral solution of ferric chloride it assumes a violet tint.

Hoffmann's Test.[2]—A small amount of tyrosin is dissolved in hot water and treated, while hot, with mercuric nitrate and potassium nitrite. The solution assumes a dark-red color and yields a voluminous red precipitate.

Separation of the Leucin.—After filtering off the tyrosin from the ammoniacal solution (see Separation of Tyrosin, above) the residual fluid is concentrated to the point of crystallization and treated with a little alcohol. This will take up the leucin. The alcoholic extract is allowed to evaporate and the residue examined with Scherer's test.

Scherer's Test.[3]—When leucin is treated upon platinum foil with nitric acid, a colorless residue is obtained which, upon the application of heat and the addition of a few drops of a solution of sodium hydrate forms a droplet of an oily fluid which does not adhere to the platinum.

Hofmeister's Test.[4]—A small amount of leucin dissolved in water causes a deposit of metallic mercury when heated with mercurous nitrate.

LITERATURE.—Frerichs, Wien. med. Woch., 1854, vol. iv, p. 465. Schultzen u. Riess, Charité Annal., vol. xv. Pouchet, Maly's Jahresber., 1880, vol. x, p. 248. Irsai, ibid., 1885, vol. xiv, p. 451. Prus, ibid., 1888, vol. xvii, p. 345. Fränkel, Berlin. klin. Woch., 1878, vol. xv, p. 265.

Xanthin crystals (Fig. 158) are very rarely observed in urinary sediments, and, so far as I have been able to ascertain, the case observed by Bence Jones[1] is the only one on record. Care should be had not to confound colorless crystals of uric acid with xanthin. Xanthin is readily soluble in ammonia. It can be identified by means of Strecker's test (which see).

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[1] Liebig's Annal., 1852, vol. lxxxii, p. 251.
[2] Ibid., 1857, vol. lxxxvii, p. 124.
[3] Jour. f. prak. Chem., 1887, vol. lxxix, p. 410.
[4] Liebig's Annal., 1877, vol. cxxxix, p. 6.

Clinically, xanthin sediments are of interest only in so far as this substance may give rise to the formation of calculi.

Soaps of Lime and Magnesia.—v. Jaksch has pointed out that in various diseases crystals may be found which "closely" resemble tyrosin in appearance, and pictures such crystals (Fig. 159), which from their behavior toward reagents he is inclined to regard as calcium and magnesium salts of certain higher fatty acids.

Should doubt arise, the question may be readily decided by a chemical examination. (See Tests for Tyrosin and Fatty Acids.)

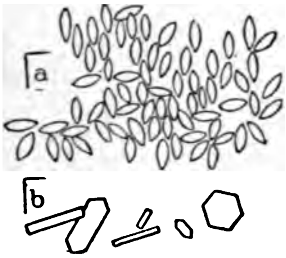

FIG. 158.—*a*, crystals of xanthin (Salkowski); *b*, crystals of cystin. (Robin.)

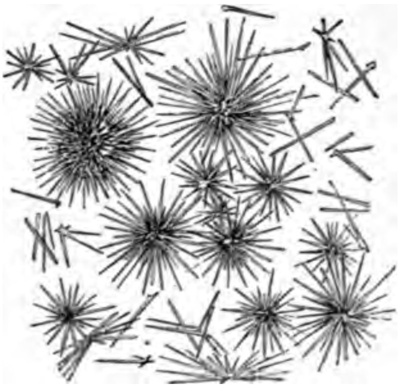

FIG. 159.—Lime and magnesium soaps. (v. Jaksch.)

Bilirubin (Hematoidin) crystals in the form of yellow or ruby-red rhombic plates or needles, as well as amorphous granules, have been seen in the urine in rare cases. They are easily soluble in alkalies and chloroform, but not in ether. When treated upon a slide with a drop of nitric acid a green ring will be seen to form around them (Gmelin's reaction).[2] Such crystals have been found either free or embedded within cells or tube casts, in cases of scarlatinal nephritis, the nephritis of pregnancy, in granular atrophy, amyloid degeneration of the kidneys, in icteric urines and in carcinoma of the bladder, of which latter condition they have been regarded by some as pathognomonic.

Fat.—When small, strongly refractive globules of fat, which may be readily recognized by their solubility in ether, are observed either floating on the urine or held in suspension, it is necessary to ascertain first of all whether such fat may not be present accidentally, owing to the use of a bottle or vessel not absolutely clean, or previous catheterization, etc. The diagnosis *lipuria* should only be made when all possible precautions have been taken to exclude the *accidental* presence of this substance. True lipuria—*i. e.*, an elimination of fat usually in the form of droplets floating on the urine—has been noted in various cachectic conditions, in cases of heart disease, affections of the pancreas and liver, in gangrene and pyemia, in diseases of the bones, especially following fractures, in diseases of the joints, in diabetes, and notably in chyluria. Fat has also been observed in the urine following the ingestion of large amounts of cod-liver oil and inunctions with fats and oils.

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[1] Chem. Centralbl., 1868, vol. xiii.

[2] Kussmaul, Würzburger med. Zeit., 1863, vol. iv, p. 64. Ebstein, Arch. f. klin. Med., 1879, vol. xiii, p. 115.

In fatty degeneration of the kidneys (nephritis, phosphorus poisoning, etc.), droplets of fat may be seen in the epithelial cells and tube casts. This, however, does not constitute lipuria. The nature of the droplets may be recognized by their solubility in ether, benzol, chloroform, carbon disulphide, xylol, etc., and by the fact that they are colored black when treated with a 0.5 to 1 per cent. solution of osmic aid, and red when a drop of tincture of alcanna is added to the specimen. A convenient method of demonstrating the presence of fat is also the following: A few cubic centimeters of the urine are mixed with an equal volume of 96 per cent. alcohol and a concentrated solution of Sudan III in 96 per cent. alcohol. The sediment which collects is then examined under the microscope; the excess of stain is removed by allowing a few drops of 60 or 70 per cent. alcohol to run under the cover-slip and removing it with filter paper placed at the edge of the preparation. The fat droplets are thus colored a vermilion red. Free fat can, of course, be demonstrated in the same manner. (See also Lipuria.)

Sediments Occurring in Alkaline Urines. Basic Phosphate of Calcium and Magnesium.—The most common sediments observed in alkaline urines consist of amorphous phosphates of calcium and magnesium. They are usually as abundant as the urate sediments which have been described, but may be distinguished from these by the fact that they do not dissolve upon the application of heat, but disappear upon the addition of acetic acid, and are never colored. In this manner it is also easy to distinguish such a sediment from one due to pus, with which it might possibly be confounded at first sight. Upon microscopic examination a drop of the sediment will be seen to contain innumerable transparent granules scattered over the entire field, and closely resembling those of urate of sodium.

Phosphatic sediments are observed, as mentioned elsewhere, whenever the reaction of the urine is alkaline, whether this be owing to the presence of fixed alkali or to ammoniacal fermentation. They also result if a faintly acid, faintly alkaline, or amphoteric urine is boiled.

Neutral Calcium Phosphate.—These crystals may be found in alkaline, amphoteric, and feebly acid urines, but are not very common. They are at times of large size, but more commonly acicular, occurring either singly or united in a star-like manner (Fig. 160). They are colorless, readily soluble in acetic acid, and insoluble in warm water, so that they can be easily distinguished from uric acid.

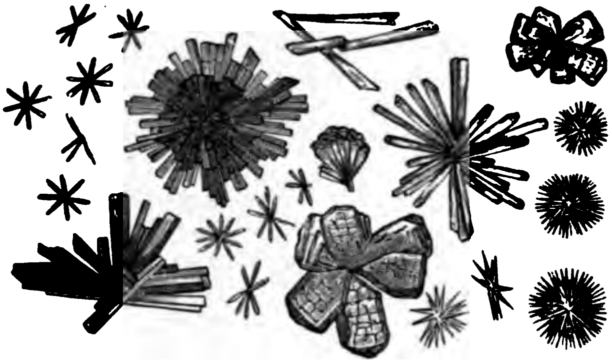
FIG. 160.—Crystalline phosphates. (Finlayson.)

Basic magnesium phosphate crystals occurring in the form of large, highly refractive plates (Fig. 161), are at times seen in alkaline, neutral, or faintly acid and highly concentrated urines. They are readily recognized by treating a drop of the sediment upon a slide with a drop of ammonium carbonate solution (1 to 4), when the crystals become opaque and their edges assume an eroded aspect. In acetic acid they dissolve with ease and may then be reprecipitated by means of sodium carbonate. They are uncommon.

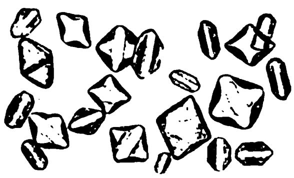
FIG. 161.—Basic magnesium phosphate crystals. (v. Jaksch.)

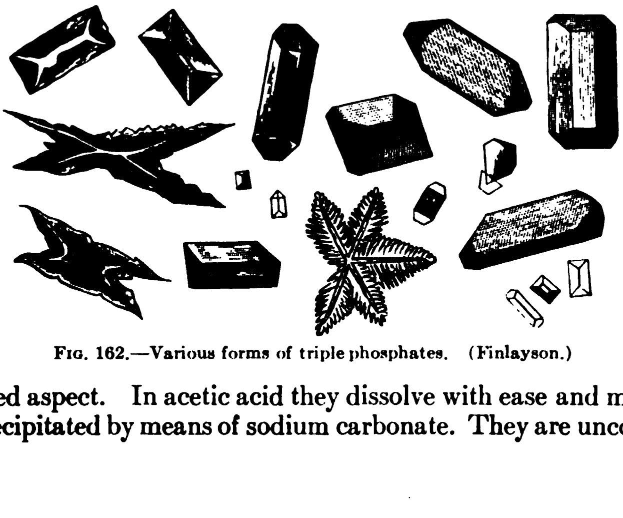
FIG. 162.—Various forms of triple phosphates. (Finlayson.)

Ammonio-magnesium phosphate, usually spoken of as triple phosphate, crystallizes in large prismatic crystals of the rhombic system; it is most abundantly observed in alkaline urines, but may also occur in feebly acid specimens. Of the various forms which may occur, that resembling the lid of a German coffin is the most characteristic (Fig. 162). At times these crystals attain a large size; very small specimens, however, also occur which may be mistaken for oxalate of calcium, but from these they are distinguished by the ease with which they dissolve in acetic acid.

Here, as elsewhere, it should be remembered that no conclusions as to the amount actually eliminated can be drawn from a microscopic examination and the diagnosis "phosphaturia" should be based only upon the results of a quantitative analysis.

The continued elimination of a turbid urine, the turbidity of which is referable to phosphates, is notably observed in certain neurasthenic individuals with a predominance of cerebral symptoms. Very curiously the phosphaturia is not influenced by diet.

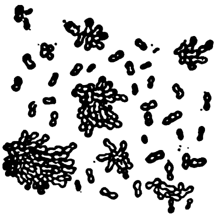

Fig. 163.—Calcium carbonate crystals.

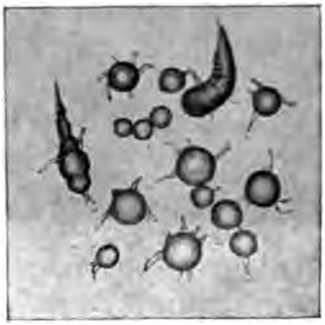

Fig. 164.—Ammonium urate crystals.

Calcium carbonate frequently occurs in alkaline urines, and appears under the microscope in the form of minute granules, occurring singly or arranged in masses; dumb-bell forms are also seen (Fig. 163). They may be recognized by the fact that they readily dissolve in acetic acid, with the evolution of gas.

Ammonium urate is observed only in urines which are undergoing ammoniacal decomposition. Its presence should always call for a careful investigation in order to ascertain whether this has taken place after the urine has been voided or before. (See Reaction.)

The salt occurs in the form of brownish, spherical bodies of variable size, which are sometimes composed of delicate needles, while at others they are amorphous, but may be beset with prismatic spicules. (thornapple forms). They are not easily mistaken for any other substance which may be present in urinary sediments (Fig. 164).

monium urate is characterized, moreover, by its solubility in tic and hydrochloric acids, and by the subsequent separation of mbic crystals of uric acid.

ndigo in the form of delicate blue needles, arranged in a stellate nner or in plates, visible only with the microscope, is rarely seen. an amorphous condition, however, it may be met with in almost ry decomposed urine, occurring in the form of small granules l sometimes staining the morphological elements that may be sent a distinct blue. Sediments presenting a bluish-black color e noted in the time of Hippocrates already, and have been cribed since by numerous observers, but the nature of the color-matter has only been determined within the last fifty years. nically, the occurrence of indigo in the urine is of interest, as al calculi have been observed which consisted almost entirely of substance. But little is known of the causes which give rise its appearance in the urine, but there can be no doubt that its urrence is referable to the action of certain microörganisms upon nary indican (which see).[1]

Organized Constituents of Urinary Sediments.

pithelial Cells (Fig. 165).—Bearing in mind the fact that desquamative processes are constantly going on in the epithelial lining of various cavities and channels of the body, one should expect to l in every urine representatives of the different forms of epithelium m the Malpighian tufts down to the meatus urinarius. To a cern extent this actually happens, and cells apparently derived from meatus, the urethra, bladder, ureters, and pelvis of the kidneys y be met with in almost every specimen, although it is often diffit to tell the origin of the individual cells. Bizzozero even claims t it is impossible to distinguish between the cells of the bladder and se of the meatus and renal pelvis, while as a class they may be erentiated in most cases from the cells of the urethra, the ureters, prepuce of the male, and the vulva and vagina of the female. lls from the uriniferous tubules are seldom seen in normal urines. The number of epithelial cells occurring in urinary sediments under ysiological conditions is small, and the presence of large numbers y hence always be regarded as abnormal. Their appearance is luenced by the reaction of the urine, an alkaline or neutral urine sing them to swell and to appear larger and rounder than in acid nes. As has been mentioned, the cellular type is practically the ne, moreover, in the bladder, ureters, and pelvis of the kidneys. As has already been stated it may be very difficult to determine the origin of single epithelial cells, or even of groups of cells, by examining these *per se*. But not infrequently other findings may lead to their proper classification and interpretation.

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[1] v. Jaksch, Prag. med. Woch., 1892, vol. xvii, p. 602.

Generally speaking three forms of epithelial cells may be found in urinary sediments, viz.:

1. Round cells.
2. Conical and caudate cells.
3. Flat cells.

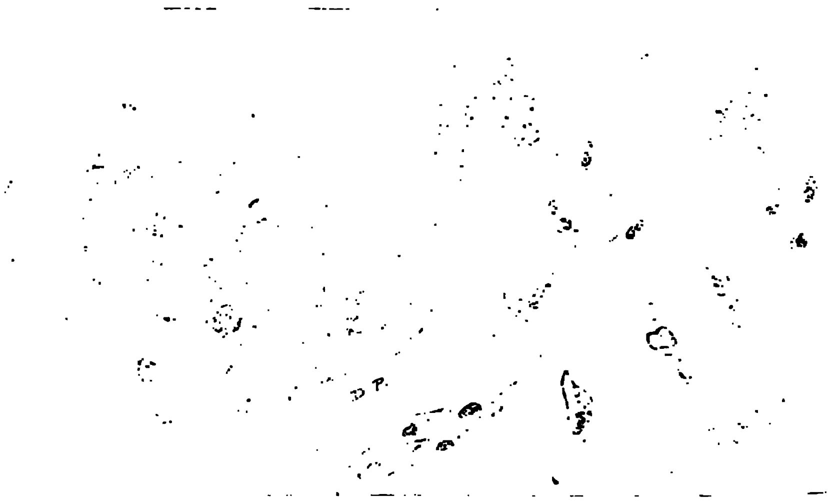

FIG. 165. Urinary epithelium.

Round cells may be derived from the uriniferous tubules or the deeper layers of the mucous membrane of the pelvis of the kidneys. They are somewhat larger than pus corpuscles and may be distinguished from these by the presence of a large, well-defined nucleus, which is readily visible as such, while in pus cells it becomes distinct only upon the addition of acetic acid, and is, moreover, multiple. Whenever such cells are found adhering to urinary casts, it is clear that they represent the glandular elements proper of the kidneys. As similar cells are found in the male urethra, confusion may arise. Should albumin be present, the cells are probably of renal origin. The presence of such cells in large numbers together with pus, in the absence of tube casts and albumin beyond traces, will usually indicate the existence of a simple pyelitis, particularly if round cells are found joined in a shingle-like manner. Should the pyelitis be associated with a nephritis, tube casts and albumin in larger amounts will at the same time be present. In such cases it may be impossible to determine the origin of the cells, excepting of such that may adhere to casts.

In simple circulatory disturbances affecting the renal parenchyma no special abnormalities can be discovered in the structure of the cells, while in fatty degeneration of the kidneys they will be seen to contain fatty particles in greater or less abundance. At other times they are markedly granular and occur in fragments.

Conical and **caudate cells** are mostly derived from the superficial layers of the pelvis of the kidneys, and are hence seen in large numbers in cases of pyelitis. Similar cells, however, are also found in the neck of the bladder.

Flat cells may come from the ureters, the bladder or the genitals. Large polygonal cells provided with single distinct nuclei and a more or less markedly granular protoplasmic zone about the nucleus are usually derived from the external genitals. Many such cells are more or less broken down and distorted. The surface cells from the bladder and ureters are less apt to show evidence of injury or degeneration, and are on the whole smaller. Surface epithelial cells from the vagina are mostly fusiform in shape and very commonly show an irregular, warped outline. Often they are seen in large plaques. Other more or less rounded forms are derived from the deeper layers of the mucosa. Irregular or conical cells, often provided with one or more protoplasmic processes, likewise come from the lower layer of the mucosa of the bladder and ureters.

In alkaline urines undergoing bacterial decomposition it is common to meet with large surface epithelial cells from the external genitals which are literally one mass of bacteria.

LITERATURE.—Bizzozero, loc. cit. Eichhorst, Lehrbuch d. physikal. Untersuch. inn. Krankheit., 2d ed., p. 336, Braunschweig.

Leukocytes.—Leukocytes are encountered in only very small numbers in normal urines. A marked increase should, hence, always be regarded as indicating the existence of disease somewhere in the urinary tract, excepting in females, where their presence may be owing to an admixture of leucorrheal discharge. In that case the source of the pus will generally be recognized by the simultaneous occurrence of pavement epithelial cells of the vaginal type in correspondingly large numbers. In doubtful cases the urine should always be obtained with the catheter, care being taken to thoroughly cleanse the vulva before the introduction of the instrument.

Occasionally the pus is derived from a neighboring abscess that has opened into the urinary passages.

The amount of pus which may be found in urines is most variable. On the one hand, deposits several centimeters in height are not uncommon, and closely resemble deposits of phosphates, for which they are indeed frequently mistaken. On the other hand, it may only be possible to discover the presence of pus by means of the microscope, which should be employed in every case.

The appearance of the pus corpuscles varies in different cases. In acid urines their form is usually well preserved, and in feebly alkaline and neutral specimens it may even be possible to observe ameboid movements when the slide is carefully warmed. In alkaline urines, however, they usually swell up and become opaque, so that it is impossible to discern a nucleus unless they are treated with acetic acid. At other times, and particularly when pus has remained long in the body, it may be almost impossible to make out a nucleus, and in extreme instances nothing but a mass of granular and fatty detritus is left.

While with a certain amount of experience it is hardly likely that a sediment of pus will be mistaken for anything else, it should be remembered that if pus is exposed to the action of ammonia or an ammonium salt the pus corpuscles become disintegrated. In such cases, as in old, neglected instances of cystitis, in which ammoniacal decomposition of the urine has taken place in the bladder, a deposit may be obtained which microscopically resembles mucus, and in which pus corpuscles may not even be demonstrable with the microscope. The sediment escapes as a gelatinous, slippery mass when the urine is poured from one vessel into another. Recourse must then be had to certain chemical tests, as a pyuria might otherwise be overlooked. To this end the following procedure, suggested by Vitali,[1] may be employed:

The urine, after having been acidified with acetic acid, is filtered, and the contents of the filter treated with a few drops of tincture of guaiacum which has been kept in the dark, when in the presence of pus the filter paper is colored a deep blue.

A solution of iodopotassic iodide may be employed in less extreme instances. A drop of this solution is added to a drop of the sediment upon a slide, when the pus corpuscles, owing to the presence of glycogen, are colored a dark mahogany-brown, while epithelial cells, with certain forms of which they might possibly be mistaken, assume a light-yellow color.

Donné's pus test is based upon the fact that the transformation of pus ino a gelatinous, mucus-like mass, observed in cases of cystitis, owing to the action of ammonium carbonate, may also be artificially produced by the addition of a small piece of caustic soda and stirring, when in the presence of pus in small amounts the liquid becomes mucilaginous and ropy, while a gelatinous mass is obtained if it is abundant.

Müller's Modification of Donné's Test.—5 to 10 c.c. of urine are treated drop by drop with official sodium hydrate solution, shaking thoroughly after the addition of each drop. If then the tube is observed, it will be noted that the bubbles of air can rise only very slowly through the viscid fluid or in the presence of fair amounts of pus may remain stationary altogether. A positive reaction is still obtained from 1200 pus cells to the cb. mm.

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[1] Maly's Jahresber., 1890, vol. xviii, p. 326.

From a clinical point of view it is important to establish the source of the pus in every case of *pyuria*. This may at times be difficult, but the following data will be found of value in a differential diagnosis:

1. In disease affecting the renal parenchyma the amount of pus, as a rule, is small, except where a large abscess located in the kidney structure proper has burst into the pelvis of the kidney.

In uncomplicated cases it is a comparatively easy matter to recognize the renal origin of the pus, as other constituents, such as renal epithelial cells, and tube casts, are usually present at the same time, and, as was noted in the case of renal epithelial cells, leukocytes are frequently found adhering to the tube casts, and at times apparently compose these entirely, when they are spoken of as *pus casts*. (See Casts.) In nephritis, according to Bizzozero, the number of pus corpuscles stands in a direct relation to the intensity and acute character of the morbid process, the greatest number being found in cases of acute nephritis, while in chronic forms their number is usually insignificant. Whenever in the course of a chronic nephritis large numbers of pus corpuscles appear, they may be regarded as indicating either an acute exacerbation of the disease or a complicating inflammation of some portion of the urinary tract. In such cases errors may be guarded against by observing the number and character of the epithelial cells present at the same time, when it will often be found that what at first sight appears as an acute exacerbation of a chronic process, judging from the number of pus corpuscles, is in reality a secondary pyelitis, ureteritis, or cystitis.

In cases of simple renal hyperemia pus corpuscles never occur in notable numbers.

2. In pyelitis the amount of pus may vary considerably, and at times even perfectly clear urine may be voided. This is probably owing to the fact that the ureter of the affected side, if the disease is unilateral, becomes obstructed temporarily, when suddenly large quantities may appear again. The diagnosis of pyelitis is often difficult, and should be based not only upon the condition of the urine, but upon the clinical symptoms as well. Very significant is the fact that the urine in pyelitis is usually acid. A careful examination of the epithelial elements may also be of value, and should never be neglected. Bacteria in large numbers are generally present.

In renal tuberculosis pus appears very early, but the amount may be extremely variable. Sometimes only a few leukocytes are seen, while at other times it may amount to one-fourth and even one-half of the urine by volume. As a rule the pyuria is constant, but cases are seen where for months and even years the urine may be almost clear and the condition is much improved. It should be remembered, however, that the passage of apparently normal urine may merely indicate that the other ureter is blocked.

When pyelitis is associated with nephritis it may at times be almost impossible to determine the origin of the pus; but if the rule set forth above is remembered, that in chronic nephritis the number of leukocytes is small, it is not likely that a pyelitis will be overlooked, particularly if the clinical symptoms are taken into consideration.

Matters may become still more complicated when a cystitis is accompanied by a pyelitis or a pyelonephritis. Catheterization of the ureters should then be resorted to, and it is highly desirable that this most valuable method of diagnosis should become common property as soon as possible. Fischl regards the presence of cylindrical masses composed of pus corpuscles, formed in all probability in the papillary ducts, as highly characteristic of pyelitis.

3. A pyuria referable to ureteritis can hardly be diagnosticated from the appearance of the urine, and in suspected cases catheterization of the ureters should be resorted to, which will probably throw light upon the question.

4. In mild cases of cystitis pus may be altogether absent, while in the more severe forms its presene is constant. In cystitis the largest amounts, referable to disease of the urinary organs, are observed, and are exceeded only in those rare conditions in which a neighboring abscess has suddenly opened into the urinary passages.

As the urine in cystitis is commonly alkaline, and always so in the more severe forms, the alkalinity being due to ammoniacal fermentation, it may happen, owing to the disintegrating action of the ammonium carbonate upon the pus corpuscles, that these may not be demonstrable with the microscope, and that a gelatinous mucoid sediment appears instead, which escapes from the vessel *en masse* when the urine is poured out. The chemical tests for pus, described above, must then be employed.

5. In urethritis pus may be present in the urine in considerable amounts. The source of the pus is recognized by the fact that a drop may be manually expressed from the urethra, particularly in the morning upon awaking. Mucoid gonorrheal threads,—the "Tripperfäden" of the Germans,—which are largely composed of pus corpuscles, will almost always be detected in the urine in such cases. In order to distinguish between a simple urethritis and a urethritis complicated with cystitis, the urine should be obtained in two portions and allowed to settle. In simple urethritis affecting the anterior portion of the urethra the first specimen is cloudy, while the second one is clear. If the urethritis, however, has extended to the neck of the bladder, in the absence of cystitis, the first portion will, of course, be cloudy, while the second may present a variable appearance, being clear at times and cloudy at

others. This phenomenon is explained by the fact that a portion of the pus contained in the posterior portion of the urethra has found its way into the bladder. A cystitis may, however, be excluded by the acid reaction of the second specimen, and the fact that the latter is never so cloudy as the first. In cases of urethritis complicated with a purulent cystitis the second portion of the urine contains at least as much pus as the first, and usually more, owing to the fact that the pus (which is heavier than the urine) falls to the floor of the bladder, in which case also the last drop passed will often be found to be pure pus. The reaction of the urine, moreover, will then be generally alkaline.

6. A sudden elimination of large quantities of pus with a urine which up to that time has presented a normal or nearly normal appearance may almost always be referred to rupture of an abscess into the urinary passages. Exceptions to this rule have been noted in rare instances in which large amounts of pus suddenly appeared, the origin of which could not be demonstrated upon postmortem investigation. Whether such a phenomenon, as v. Jaksch suggests, is dependent upon "unusual conditions favoring diapedesis" remains an open question.

Enumeration of the Pus Corpuscles in the Urine.—In order to determine the relation existing between the degree of pyuria and albuminuria, as well as to watch the progress of an individual case, an enumeration of the number of pus corpuscles is at times necessary. To this end a specimen of the urine is thoroughly shaken and the number of corpuscles contained in one cubic millimeter ascertained with the aid of the hemocytometer (Simon's ruling). Dilution with a 3 per cent. solution of common salt is necessary if a preliminary examination has shown the presence of more than 40,000 corpuscles per cb. mm. A dilution of five times is usually sufficient.

Some of the results which have thus been obtained are extremely interesting. In cases of mild cystitis 5000 pus corpuscles are found on an average in the cubic millimeter; in cases of moderate severity from 10,000 to 20,000; while in severe cases 50,000 and even more may be seen. In one case of cystitis complicating carcinoma of the bladder Hottinger obtained 152,000 per cb. mm. In the presence of less than 50,000 a mere trace of albumin is found, and with 80,000 to 100,000 only 1 pro mille is referable to this source.[1]

Red Blood Corpuscles.—The presence of red blood corpuscles in the urine, constituting the condition usually spoken of as *hematuria*, is observed only in pathological conditions, and is, in contradistinction to hemoglobinuria (which see), a relatively common occurrence.

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[1] R. Wunderlich. *Ueber d. Werth d. Zählung d. weissen Blutkörperchen im Harn, etc.*, Diss., Würzburg. 1895.

Urine containing blood corpuscles in notable numbers presents a color which may vary from a bright red to a dark brown verging upon black. Upon standing, a sediment of a corresponding color is obtained in which distinct coagula of variable size are at times seen.

If the urine should contain only a small number of red corpuscles, however, no deviation from its normal appearance will be noted, and the diagnosis of hematuria can then only be made with the microscope, which should be employed in every case. The appearance of the red corpuscles varies greatly, being influenced especially by the length of time during which they have remained in the urine. In cases of hematuria of urethral or vesical origin it will be found that they have mostly retained their normal appearance fairly well, or have become crenated, when they may be recognized without difficulty. In cases, on the other hand, in which the corpuscles have remained in the urine for a longer time, as in hematuria of renal origin, the inexperienced will frequently be puzzled by the presence of bodies of the size of red corpuscles, or somewhat smaller, which are entirely devoid of coloring matter, and appear as faint, transparent rings, often presenting a double contour, and in which no nucleus can be discovered. These formations are red blood corpuscles from which the hemoglobin has been dissolved. They are spoken of as *blood shadows*. Chemical tests are rarely necessary, but may be employed if doubt should arise.

Clinically it is, of course, all-important to determine the source of the blood. This may at times be accomplished without much difficulty by a urinary examination, but at other times it may almost be impossible, when the clinical symptoms and physical signs must be taken into consideration.

1. Hematuria of urethral origin, due to urethritis, prostatitis, or traumatism incident to catheterization, for example, is a common event, and readily diagnosticated, as in such cases blood either escapes of itself from the urethra or it may be squeezed out manually. The last portion of the urine voided, moreover, will always be found free from blood, unless it is referable to disease of the neck of the bladder, when the blood appears only toward the end of micturition, or at least more markedly then than in the beginning.

2. The diagnosis of vesical hematuria is not always easily made. It should be remembered, however, that the blood corpuscles here present a normal appearance, as has been mentioned, unless ammoniacal decomposition is occurring in the bladder, in which case blood shadows are seen in large numbers. The blood, moreover, is less intimately mixed with the urine than in cases of renal hematuria, so that the corpuscles rapidly settle after the urine has been passed. Blood clots of an irregular form and considerable dimensions can only be of vesical origin. A careful examination for the presence

of any other morphological constituents which may be observed in urinary sediments, when considered in conjunction with the clinical symptoms, will usually lead to a correct diagnosis so far as the seat of the hemorrhage is concerned. Hematuria of vesical origin may be due to numerous causes, among which may be mentioned hemorrhagic cystitis, stone, tuberculous ulceration, malignant growths, papilloma, traumatism, the presence of parasites, and, more rarely, rupture of varicose veins in the bladder. In determining the cause of the hemorrhage in a given case more reliance should be placed upon the clinical history and a direct examination of the bladder, than upon the urinary examination.

3. In hematuria of ureteral origin characteristic blood coagula, corresponding in diameter and form to the ureters, are occasionally seen. Their presence, however, does not necessarily indicate that the blood has come from the ureters; more frequently the hemorrhage will be found to be due to disease of the pelvis of the kidney.

4. The diagnosis of hemorrhage into the pelvis of the kidney must be based upon the clinical symptoms taken in conjunction with the results of a urinary examination. In nephrolithiasis only a small number of red corpuscles is usually found, which is important from the standpoint of differential diagnosis. In renal tuberculosis hematuria is one of the most important symptoms and not infrequently the first which attracts the attention of the patient. The amount is variable; sometimes the bleeding is microscopic, while in others almost pure blood is passed. It is usually intermittent, the periods of bleeding lasting from one hour to several weeks, the average being three days. Late in the disease it is usually less in amount, but apt to be almost continuous. As a rule the urine and blood are intimately mixed. Clotting, however, may occur in the bladder and the pelvis of the kidney.

5. Hematuria of purely renal origin is of common occurrence, and may be due to numerous causes. In simple hyperemic conditions of the organs and in hemorrhagic nephritis the passage of smoky-looking urine containing blood corpuscles, usually in large numbers, is thus a fairly constant symptom. In chronic nephritis the number of the red corpuscles may be taken to indicate the intensity of the morbid process. Hematuria may also be due to renal abscess, renal tuberculosis, malignant growths, stone, and, in rare instances, to aneurysm and embolism of the renal artery, thrombosis of the renal vein, papilloma of the pelvis, etc. In the malignant forms of the acute infectious diseases, such as smallpox, yellow fever, malaria, etc., in scurvy, hemophilia, and purpura, in leukemia, filariasis, and distomiasis, renal hematuria is common. It is also observed in cases of poisoning with turpentine, carbolic acid, cantharides, and has recently also been observed in several convalescents from typhoid fever while under

treatment with urotropin; the hematuria ceased with the discontinuance of the drug.[1]

6. **Functional Hematuria.**—An idiopathic form of hematuria has also been described, in which hemorrhage from the kidneys occurs without apparent cause. This is relatively common. Senator speaks of it as renal hemophilia. It has repeatedly led to errors in diagnosis and more particularly in connection with renal tuberculosis, as it also is usually unilateral. The amount of blood is very variable, sometimes only microscopic, at others excessive. I have seen three cases of this kind in which no lesion existed which could be made responsible for the hemorrhage. In all three the attacks of hematuria were associated with anachlorhydria, while normal values were found between the attacks. Two of the patients were males, and undoubtedly neurasthenics. The third was a hysterical, chlorotic female, in whom hematemesis, pulmonary hemorrhages, and melena were also at times observed.

Hematuria of renal origin is usually recognized without much difficulty, as in such cases tube casts bearing red blood corpuscles, and at times apparently consisting of these altogether, as well as numbers of renal epithelial cells, will usually be found upon examination. The blood, moreover, is intimately mixed with the urine, and the individual corpuscles have mostly lost their hemoglobin and appear as mere shadows. The clinical history should, of course, always be taken into consideration, especially in determining the primary cause of the hemorrhage.

Urine containing red blood corpuscles is always albuminous, so that it may sometimes be difficult to decide in a given case whether the albumin found is due solely to the presence of blood or whether the hematuria is complicated with an albuminuria *per se*. Frequently it is possible to arrive at some conclusion by comparing the amount of albumin with the number of the red corpuscles, the presence of a large amount of the former in the presence of only a small number of the latter indicating that the albumin is not altogether due to the blood. At other times it is impossible to gain information in this manner, when the only expedient left is to determine the quantity of albumin and of iron separately, and to ascertain whether the amount of iron found is sufficient to combine with that of the albumin. As a rule, however, the presence of serum albumin, aside from that contained in the blood of the urine, may be inferred whenever tube casts are present, although the amount can only be estimated approximately in this manner.

Tube Casts.—In various pathological conditions, and it is claimed even in health, curious formations are seen in the urine, which represent molds of different portions of the uriniferous tubules. To these the term *tube casts* or *urinary cylinders* has been applied. The term "tube casts," however, is not altogether appropriate, as it is applicable to only one great division of such formations—*i. e.*, to those consisting of a uniform, transparent, gelatinous matrix, to which other elements, such as epithelial cells, red blood corpuscles, leukocytes, and salts in a crystalline or amorphous form, may accidentally have become attached—*the tube casts proper.*

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[1] Griffith, Milligan, and Forbes, Brit. Med. Jour., June 29, 1901.

From these the so-called "pseudocasts" must be differentiated, a pseudocast being characterized essentially by the absence of a uniform matrix. Closely related apparently to the true casts are the so-called cylindroids—*i. e.*, band-like formations which resemble the former in appearance, and like these may carry various morphological elements. It is thus necessary to distinguish between true casts, pseudocasts, and cylindroids. Of these, the true casts are the most important. They may be divided into hyaline and waxy casts, the two forms being readily differentiated by the fact that the former readily dissolve in acetic acid, while the waxy casts are either not affected at all by this reagent, or, if so, at least not so rapidly. The latter, moreover, are more strongly refractive, to which property their waxy appearance is due; their color is slightly yellow or yellowish gray, while the hyaline casts are colorless and usually very pale and transparent.[1]

Mode of Examination.—Unless a urine can be examined within a few hours afer being voided, it is well to add a small amount of chloroform, so as to guard against bacterial decomposition. The use of conical glasses is unsatisfactory, and I find it more convenient to keep the urine in well-stoppered bottles. Preserved with chloroform it will keep almost indefinitely. Where a centrifugal machine is available the specimen can, of course, be examined at once. As soon as a sufficient amount of sediment has been obtained, a few drops are spread on a slide and examined, *uncovered, with a low power. It is essential, however, to make use of the flat mirror and to avoid a bright light. If this is borne in mind, no difficulty whatever will be found in demonstrating even the most hyaline specimens, though they may be present in very small numbers.* In many textbooks on urinary analysis the writers speak of the difficulty attending the search for hyaline casts, and the advice is frequently given to color the preparations with a drop of a dilute aqueous solution of iodopotassic iodide, or of some other staining reagent, such as gentian violet, picrocarmine, methylene blue, or osmic acid. This is unnecessary if the directions just given are strictly followed. If a *bright* light is used, however, I am willing to admit that even the most experienced examiner may be unsuccessful in his search.

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[1] Rovida, see J. Moleschott, Untersuchung. z. Naturlehre d. Menschen u. d. Thiere, 1867, vol. xi, I, p. 182.

For the preservation of mounted specimens the following method, devised by Krönig, may be employed, though I personally prefer to keep the urine itself and to mount a fresh specimen when necessary. A drop of the sediment, best obtained by centrifugation, is spread on a cover-glass and allowed to dry in the air. It is then placed in a 10 per cent. solution of formalin for ten minutes, rinsed in water, and stained for about ten minutes in a concentrated solution of Sudan III in 70 per cent. alcohol. The excess of stain is removed by immersion for one-half to one minute in 70 per cent. alcohol, when the specimen is counterstained with Ehrlich's hematoxylin, rinsed in water, and mounted in glycerin. Evaporation is guarded against by ringing the specimen with asphaltum. The tube casts are thus stained a more or less pronounced blue, the nuclei of the leukocytes dark blue, and any fatty granules or needles of fatty acids that may be present a bright red.

I have obtained very satisfactory results by pouring a small amount of a 1 per cent. aqueous solution of eosin into one of the tubes of the urinary centrifuge, filling up with urine and then centrifugating. The supernatant fluid is poured off and the sediment mixed with Farrant's solution; the specimens are finally ringed with asphaltum and keep for a long time. The hyaline casts appear a delicate rose, while the fatty casts are a bright vermilion and the brown, granular casts a reddish brown. Adhering granules or cells are colored a bright red.

Liebmann[1] recommends a mixture of 2 grams of methylene blue dissolved in 100 c.c. of a 10 per cent. solution of formalin. The urine is first centrifugated, the supernatant fluid is poured off, when a few drops of the reagent are poured on the sediment, and left a few minutes. The tube is filled with water, left for awhile for the salts to dissolve, then centrifugated again, when the formed elements are ready for microscopic examination.

TRUE CASTS. *Hyaline Casts* (Plate XX).—Upon careful examination it will be seen that with rare exceptions the matrix of hyaline casts is not *altogether* homogeneous, as small granules may almost always be detected embedded in or adhering to the matrix. As these granules occur in greater or less numbers, hyaline casts are spoken of as being finely granular (Plate XX), coarsely granular, finely dotted, etc. Should true morphological elements be detected, the casts are termed blood casts, epithelial casts, or pus casts (Fig. 166). It would be better, however, to add the term hyaline in every instance, so as to distinguish them from pseudo-casts, which consist of these elements entirely, and lack a uniform matrix. It would thus be proper to speak of hyaline epithelial casts,

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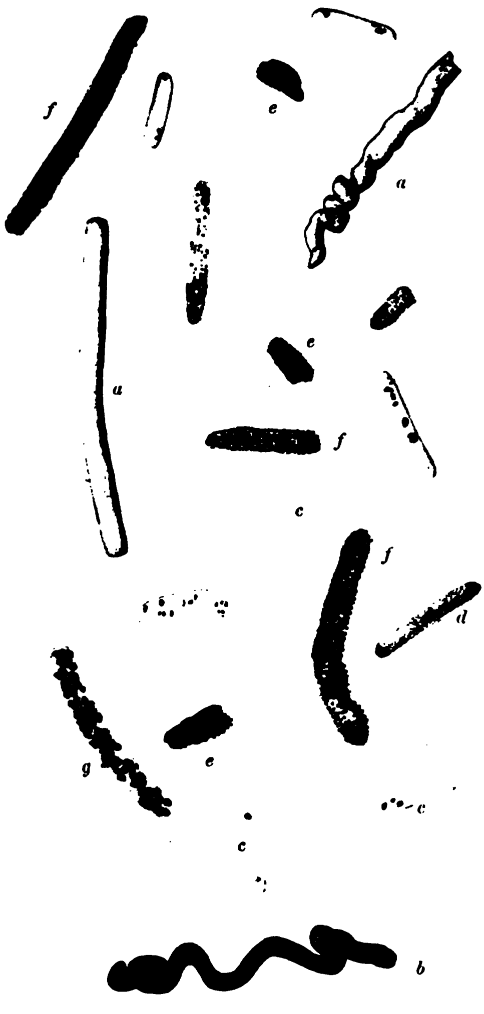
[1] Hospitalstidende (Copenhagen), July 30 to August 20, 1902. Abst. in Jour. Amer. Med. Assoc., September 20, 1902.

PLATE XX.



Casts.

a, a, **waxy casts;** *b*, **same, stained with eosin;** *c, c, c*, **hyaline casts;** *d*, **same, stained with eosin;** *e, e, e*, **brown granular casts;** *f, f*, **coarsely granular casts;** *g*, **epithelial cast;** *f*, **blood cast, stained with eosin. (Low-power picture, Leitz 3.)**

ne blood casts, etc., and to apply the collective term—compound ne casts—to these various subvarieties.

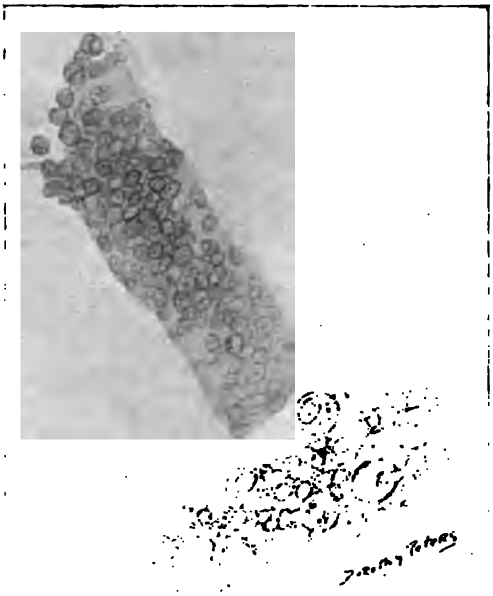


FIG. 166.—Pus and epithelial casts.

e nature of these various forms can probably always be made vithout much difficulty, and even in those cases in which the

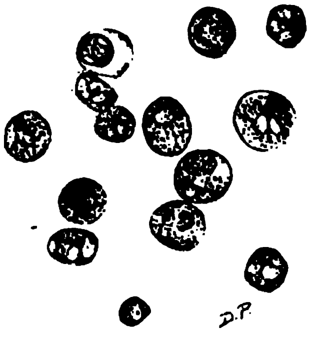


FIG. 167.—Pus cells from a urinary sediment.

ne matrix is apparently concealed beneath cellular elements it usually be possible, upon closer observation, to detect a fine boundary line at some portion of the structure. Not infrequently also the end of the cast will be seen to be more or less distinctly hyaline. In others, again, a hyaline zone may be observed along the sides of a central organized thread, so to speak, this being frequently seen in specimens which are very broad and long. Should doubt arise, however, a drop of acetic acid is added to a drop of the sediment on the slide; the acid dissolves the hyaline matrix, the organized constituents are set free, and the differential diagnosis between a pseudocast and a compound hyaline cast is thus readily established.

The length of hyaline casts varies greatly. It may scarcely exceed the breadth, on the one hand, while, on the other, although rarely, the casts may traverse the entire microscopic field. In breadth they vary between 0.01 and 0.05 mm. As a rule, the breadth of a cast is uniform throughout its entire length, but specimens are not infrequently observed in which one end tapers considerably and presents a spirally twisted appearance. This may be so marked that the entire cast appears transversely striated. It is generally supposed that this results from the adhesion of one end of the cast to the walls of a tubule, the lumen of which it does not fill, so that the free end becomes twisted in the downward course. A dichotomous branching of one end is also at times seen in very broad hyaline specimens.

Fat globules are frequently found upon hyaline casts and are probably derived from degenerated epithelial cells. When present in large numbers such casts are termed fatty casts. The globules are soluble in ether and are colored red by Sudan III. (See Tests for Fat.)

Granules of melanin, indigo, and altered blood pigment may also at times be observed in casts.

Regarding the mode of formation of the hyaline casts it is now thought that the matrix is essentially an inflammatory exudate, formed through the activity of the morbidly altered epithelial cells, and subsequently coagulated in the tubules.

Brown Granular Casts.—These should not be confounded with the compound hyaline variety. They show no evidence of a hyaline matrix and on staining with eosin they are colored a deep brownish red. (Plate XX.) Unstained they appear brown. They are unquestionably composed of epithelial cells which have undergone degeneration, the residual material being then packed together in cast form. They are quite brittle and often not longer than they are broad. The true nature of these small masses can be made out by staining with eosin, when it will be seen that they stain exactly like the larger pieces that have not yet broken down.

The *waxy casts* may be divided into two groups—true waxy casts and amyloid casts; but as the latter are not necessarily indicative of the existence of amyloid degeneration of the kidneys, such a classification is of only theoretical interest. They are readily distinguished from the hyaline casts by the characteristics mentioned above—*i. e.*, their higher degree of refraction, their yellow or yellowish-gray color, and the fact that they are either not attacked at all by acetic acid or only very gradually. Their appearance suggests a much more solid object than the hyaline casts. As a rule, only small fragments are found, but in some instances very long casts are seen and occasionally I have found such long casts which were branching. Some of these casts at times present a peculiar knotty appearance. With eosin the waxy casts are colored a bright vermilion, while hyaline casts show only a pink color. Waxy casts may also contain cellular elements, crystals, and amorphous mineral matter; but, as a rule, such compound casts are not so commonly observed as are those of the hyaline variety.

As has been stated, some waxy casts give the amyloid reaction—*i. e.*, they assume a mahogany color when treated with a dilute solution of iodopotassic iodide, which changes to a dirty violet upon the addition of dilute sulphuric acid. It should be remembered, however, that this reaction in casts does not necessarily indicate the existence of amyloid disease of the kidneys, as the reaction may be absent in this condition, and present where amyloid degeneration does not exist. This curious phenomenon is usually explained by assuming that such casts have remained in the uriniferous tubules for a long time, and have there undergone certain chemical changes analogous to the so-called "amyloid metamorphosis" of old precipitates of fibrin. Frerichs has pointed out that fibrin which has remained in the uriniferous tubules for a long time becomes denser and yellowish in appearance, which would explain the fact that these casts are only with difficulty attacked by acetic acid.[1]

The waxy casts, like the brown granular casts, are ultimately supposedly of epithelial origin.

Before leaving this subject it should be stated that "cast-like" formations consisting entirely of amorphous urates are not infrequently encountered in urines, and according to Leube[2] they may be obtained from any urine if it is concentrated in a vacuum at a temperature of 37° to 39° C. Students frequently regard such formations as coarsely granular casts, an error which may be guarded against if the characteristics of hyaline casts set forth above are borne in mind. Such structures are not colored by eosin.

Bacteria (in cases of infectious pyelonephritis), hematoidin, and granular detritus frequently occur grouped in a cast-like manner; their nature is readily ascertained, as in the case of the so-called urate casts just described.[3]

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[1] Rovida, loc. cit. Kobler, Wien. klin. Woch., 1890, vol. iii, pp. 531, 557, 574, 576.

[2] Zeit. f. klin. Med., 1887, vol. xiii.

[3] Martini, Arch. f. klin. Chir., 1884, vol. xvi, p. 157. v. Jaksch, Deutsch. med. Woch., 1888, vol. xiii, Nos. 40 and 41.

Pseudocasts, consisting of epithelial cells or blood corpuscles and fibrin, are not often found in urinary sediments. The epithelial pseudocasts are probably seen only in cases of desquamative nephritis, and, unlike true casts, are hollow, the epithelium of the uriniferous tubules being thrown off *en masse*. Blood casts consist of fibrin, within the meshes of which red corpuscles are found; these either present a normal appearance or occur as shadows, owing to the fact that their hemoglobin has been dissolved. They are seen whenever extensive hemorrhage has taken place in the renal parenchyma, and are more common than the epithelial pseudocasts.

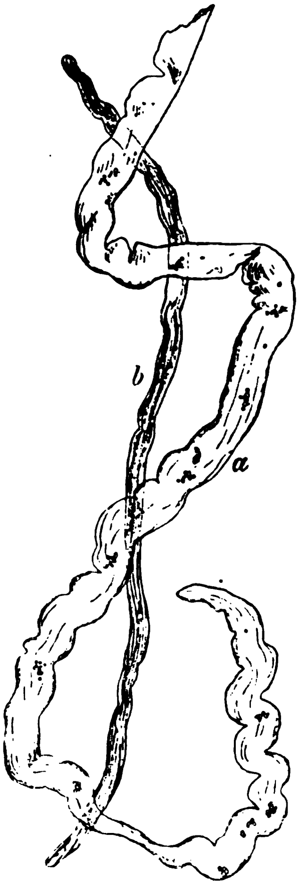

Fig. 168.—*a* and *b*, cylindroids from the urine in congested kidney. (v. Jaksch.)

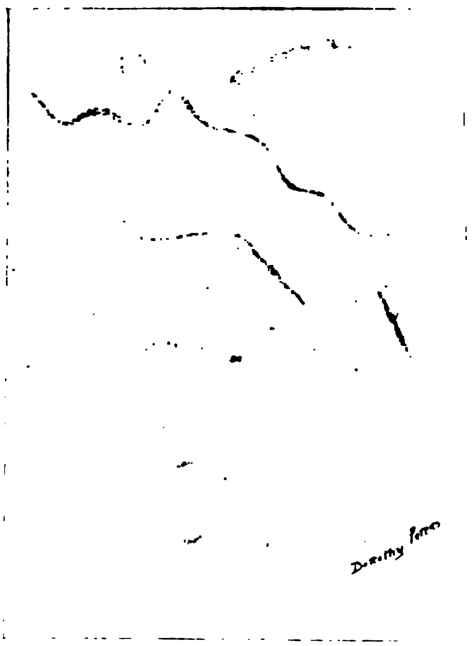

Fig. 169.—Mucous cylinders.

Cylindroids (Fig. 168) resemble hyaline tube casts somewhat in general appearance, but differ from them in being much larger and band-like. Like true casts, they have a uniform breadth, and are often beset with crystals and cellular elements, such as leukocytes, red corpuscles, and epithelial cells. They are readily dissolved by acetic acid, thus differing from the *mucous cylinders* or pseudocylinders (Fig. 169) which may be observed in any urine containing mucus; the latter probably never contain morphological or mineral constituents, and are never of uniform breadth throughout their length. The cylindroids proper are undoubtedly of renal origin and closely related to true casts; formations are indeed not infrequently seen in which a tube cast terminates in a cylindroid at one or both ends.[1]

Clinical Significance of Tube Casts.—Formerly the occurrence of tube casts in urine was held to indicate the existence of nephritis. This view has been abandoned, however, for the same reasons which led to the rejection of the idea that albuminuria invariably indicates Bright's disease (see above).

The statement is frequently made in text-books that tube casts may occur in the urine of perfectly healthy individuals, following severe muscular exercise, cold baths, etc.—in short, stimuli which may cause the appearance of albumin in apparently normal individuals. It has been indicated elsewhere (see Functional Albuminuria), however, that such stimuli cannot be regarded as "physiological" in every instance, and *the presence of tube casts in the urine similarly should be regarded as a pathological event.*[2] This, however, does not invalidate the now generally recognized fact that a small number of hyaline and granular casts can be demonstrated in the *centrifugated* urine of many people who are to all intents and purposes in good health.

It is not necessary in this connection to enumerate the various diseases in which cylindruria is observed, as they are the same as those which give rise to albuminuria; and just as a *nephrangiogenic albuminuria* is more frequently observed than a *nephritidogenic albuminuria,* so also is the presence of tube casts in the urine more frequently due to circulatory disturbances than to nephritis. In every case in which tube casts occur in the urine it may be assumed that the accompanying albuminuria is, to a certain extent at least, of renal origin.

Formerly it was thought possible to diagnosticate the character of the underlying renal disturbance from the type of casts found in the urine. This, however, is not the case. While generally speaking blood and epithelial cells are found in acute and granular casts in chronic processes, there are exceptions so numerous that it would not be safe to follow such a rule. It is remarkable to see the large number and the many varieties of casts which may be found in the urine during the first twenty-four to forty-eight hours after anesthesia, and to observe how rapidly they may disappear, no evidence remaining whatsoever that the renal parenchyma has shortly before been seriously taxed.

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[1] Bizzozero, loc. cit. Thomas, Arch. f. Heilk., 1870, vol. xi, p. 130. Pollak u. Török, Arch. f. exper. Path. u. Pharmakol., 1888, vol. xxv, p. 87.

[2] Nothnagel, Deutsch. Arch. f. klin. Med., 1874, vol. xii, p. 326. Burkhart, Die Harncylinder, 1884. Fischel, Prag. Vierteljahrschr., 1878, vol. cxxxix, p. 27.

Cabot has recently pointed out the lack of correspondence between the clinical diagnosis of renal disease, as based upon urinary examination and the pathological findings, and has given expression to what many clinicians have previously realized, viz., that neither the diagnosis nephritis nor the type of the renal disturbance can usually be made with certainty in the laboratory. My own experience has led me to the conclusion that so far as cylindruria is concerned the continued presence of hyaline and granular casts, especially of the dark-brown variety, is a symptom of greater gravity than the temporary occurrence of the other types. Hyaline casts *per se* are found under the most diverse conditions. Almost any renal disturbance, whether temporary or permanent, leads to their appearance. Their number is sometimes most remarkable, notwithstanding the fact that no permanent renal damage has been done. Finely dotted and finely granular casts are generally present at the same time.

As the granular cast is generally viewed as a hyaline cast which has been retained in the tubules for a longer time, and, as a result, has undergone changes leading to its granular appearance, it might be inferred that in many temporary disturbances this type is not found. In a general way this is true, but the *occasional* finding of granular casts only should not lead to the diagnosis of a chronic disturbance. They also can appear quite suddenly and disappear almost as rapidly.

Epithelial casts and blood casts are met with in acute processes or in acute exacerbations of chronic processes.

Waxy casts always indicate a chronic or, at least, a subacute process. The fatty casts described by Knoll and v. Jaksch "are most commonly associated with subacute or chronic inflammations of the kidney of protracted course, with a tendency to fatty degeneration of the renal tissue. Postmortem examination has shown that they form most frequently in cases of large white kidney. In some cases in which they were present, however, the organ was found to be more or less contracted; but when this was so, it was invariably far advanced in fatty degeneration." (v. Jaksch.)

It has been stated that from an examination of the renal epithelial cells it is often possible to determine whether an inflammatory process affecting the kidneys is at the same time complicated with degenerative changes. As a matter of fact, the cells found on the tube casts under such conditions no longer present a normal appearance, but are shrunken and atrophied, and in cases of fatty degeneration studded with fatty granules.

The occurrence of *pus casts* presupposes the existence of suppurative inflammation in the kidneys, while the presence of only a small number of leukocytes on hyaline casts may be observed in the ordinary forms of nephritis, and particularly in the acute form.

Cylindroids are present whenever hyaline casts are seen, and have essentially the same import. They are said to occur most frequently in the urine of children.

So far as the constancy is concerned with which tube casts occur in the urine in nephritis, it is well known that in the chronic interstitial form of the disease, they, as well as albumin, are frequently absent for a long time, so that it may only be possible to make the diagnosis from the clinical history and the physical signs. It is a well-known fact, moreover, that pathological alterations of the kidneys, particularly in men past middle age, are observed again and again in the postmortem room, where a previous examination of the urine showeed no evidence of the existence of renal disease. In the acute and subacute forms of nephritis, as well as in the ordinary parenchymatous form, tube casts are probably always found, and it would further appear that acute circulatory disturbances affecting the renal parenchyma quite constantly lead to both albuminuria and cylindruria.

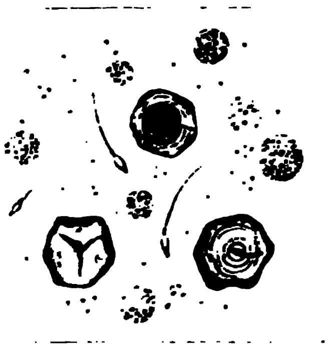

FIG. 170.—Spermatic fluid, showing spermatozoa, corpora amylacea, and lecithin corpuscles.

Within recent years attention has been repeatedly called to the occasional occurrence of cylindruria without albuminuria. Nothnagel first noticed this in a case of icterus. Lüthje observed the same after administering salicylic acid and Stewart has drawn attention to its occurrence in the early stages of chronic nephritis. I have observed the same after the administration of ether.

Spermatozoa.—Spermatozoa, for a description of which the reader is referred to the chapter on the Semen, are frequently observed in the urine of healthy adults, and are quite constantly met with in the first urine passed after coitus or nocturnal emissions, when their presence is, of course, of no significance (Fig. 170).

In females semen may be found in the urine when the external genitals have been polluted during or after coitus, as well as in the exceptional cases in which connection has been effected by the urethra. From a medicolegal standpoint the discovery of spermatozoa in the urine of women may be of great importance, but otherwise it is without significance.

In pathological conditions spermatozoa are not infrequently found in the urine. In cases of obstinate constipation, owing to pressure of hard, scybalous masses upon the seminal vesicles, a partial evacuation of semen may occur. Horowitz has pointed out that a discharge of semen may be noted in cases of peri-urethral abscess with perforation into the ejaculatory ducts, giving rise to *spermatocystitis*, the condition being due to a tight stricture of the urethra with dilatation beyond the constricted portion. I have observed a case of cystitis in which spermatozoa could almost always be detected in the urine. An operation revealed a tight stricture of the urethra and a sacculated bladder; the constant passage of semen was apparently owing to the irritating action of the ammoniacal urine. In the urine voided during and after epileptic and, more rarely, hystero-epileptic seizures spermatozoa may be found. Such an event is undoubtedly due to muscular spasm, and is identical in origin with the emission of semen observed so frequently in the death agony, and during strangulation.

In certain spinal diseases semen may be found in the urine, and Fürbringer relates a case in which, following fracture and dislocation of the vertebral column, with partial destruction of the middle dorsal cord, spermatorrhea associated with partial erection occurred thirty hours later, and continued until death, which took place after three days.

More important is the loss of semen noted in cases of true *spermatorrhea* due to venereal excesses or masturbation, when spermatozoa may be found almost constantly, and the diagnosis indeed will often be dependent upon such an observation.

So far as the question of *sterility* in the male is concerned, reliance should not be placed upon an examination of the urine, but the semen should be obtained as soon as possible after ejaculation, and examined as indicated elsewhere.

Parasites. Vegetable Parasites.—It has been shown by numerous investigations that bacteria are always present both in the male and female urethra, and that they may *at times* gain entrance to the bladder. The weight of evidence, however, is in favor of the view that the urine *intra vesicam* is under normal conditions free from microörganisms, and that bacteria which may have found their way into the bladder are rapidly killed in healthy individuals. In every urine, on the other hand, that has been exposed to the air, bacteria are always present. Whenever, then, it is desired to determine whether or not the urine of the bladder contains microörganisms, every precaution should be taken to guard against accidental contamination. To this end the following method should be employed: If the patient is a male, he is instructed to hold his urine until a fairly large amount has accumulated. The glans is then thoroughly washed with soap and water, and further cleansed with cotton soaked in mercuric chloride solution (1 to 1000). The fossa navicularis is also thorougly cleansed with the same solution. The urine is then voided under as great pressure as possible. The first portion (about 100 c.c.) is thrown away, and the second received in a sterilized vessel, when cultures should be made at once, agar or gelatin plates being inoculated with 1 or 2 c.c. of the urine. In the female the vulva is cleansed with soap and water, and the urethral aperture disinfected with bichloride solution. After then washing with sterilized water and drying with sterilized cotton the urine is evacuated through a sterilized metallic or glass catheter, and received in a sterilized vessel. Brown describes the method which is in use in Dr. Kelly's department at the Johns Hopkins Hospital as follows: The external urethral orifice being carefully cleansed with mercuric chloride solution, followed by sterile water, a sterilized glass catheter, whose external end is covered by a sterile rubber cuff, extending several centimeters beyond the end of the catheter, is introduced, the fingers of the operator being allowed to touch only the distal end of the rubber cuff. The urine is allowed to flow for a short time, when the rubber cuff is pulled off by traction on its distal end. A small amount of urine is then collected in a sterile test-tube, and the cotton plug immediately inserted. Brown states that an extended series of experiments with normal urines has shown that this method is absolutely reliable.[1]

Of the bacteria which may be found in every urine that has been exposed to the air, the *Micrococcus ureæ* is of special interest, as ammoniacal fermentation is largely due to its presence. When fermentation has commenced, it is readily recognized, occurring in almost pure culture upon the surface of the urine, mostly in the form of characteristic chains. The individual coccus is colorless and quite large, so that it may be mistaken by beginners for a blood shadow.

It is a common error to infer from the occurrence of ammoniacal decomposition very soon after micturition that this process has already begun in the bladder. It should be remembered that urine may undergo fermentation, particularly in warm weather, shortly after having been voided, and especially if the vessel employed is not perfectly clean and the urine has been exposed to the air. The diagnosis of ammoniacal fermentation in the bladder should hence only be made when the presence of ammonia can be demonstrated in the urine immediately upon being voided.

Under pathological conditions various pathogenic bacteria may be found in the urine. Their presence usually indicates the existence of definite changes in the renal parenchyma, although these changes are not necessarily of an inflammatory character. Pyogenic cocci are especially prone to settle in the kidneys, and there give rise to focal inflammations; but even in the absence of such lesions they are frequently found in the urine. In all forms of infectious nephritis an abundant elimination of bacteria may generally be observed. Von Jaksch states that in erysipelas the bacteriuria and nephritis disappear, together with the cessation of the disease, and in various suppurative processes taking place in the body the specific bacteria disappear from the urine within twenty-four to forty-eight hours after evacuation of the pus.

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[1] T. R. Brown, loc. cit.

Most interesting observations on the occurrence of bacteria in the urine of nephritic patients have been reported by Engel: 31 cases were examined. In 16 the Staphylococcus albus and aureus were found, in 8 pyogenic streptococci, in 4 the tubercle bacillus, in 5 the Bacillus coli communis, and in 1 the typhoid bacillus, while negative results were obtained in only 2 instances. In the same series Engel also found a pyogenic coccus in 17 cases. This coccus was larger than the known forms; it could be stained according to Gram's method, and did not liquefy gelatin. Intravenous injections of large numbers of the organism caused nephritis in rabbits.

In pneumonia and pneumococcus infections in general the corresponding diplococcus may be found, and in erysipelas and streptococcus infections streptococci. In scarlatina streptococci have been found in a large percentage of cases; the urine was then more often albuminous than non-albuminous.

In cases of pyelitis the colon bacillus is very frequently met with. It is usually present in pure culture, but may be associated with other organisms, notably the Proteus vulgaris and staphylococci. These latter may, however, also be met with in pure culture.

In *renal tuberculosis* the corresponding bacilli appear very early and are always present in the pus and debris. The search for them is usually very tedious, and small numbers only are found, but at times they are very numerous. To demonstrate their presence the urine is allowed to settle for twelve hours. Slides are prepared, which must be free from fat. To this end they are boiled for thirty minutes in a strong solution of caustic soda and then washed for an equal length of time in running water, after which they are wiped dry. Two drops of the sediment are placed on each one of six slides. They are placed on a frame some ten inches above a Bunsen burner, which is kept low, so as to ensure slow evaporation. When thoroughly dry they are fixed by passing through the flame of the burner and placed for five minutes in a 5 per cent. acid (HCl) alcohol to dissolve the urinary salts. After washing in water the specimens are then stained as usual. Using Gabbett's method they are stained for ten minutes with the carbol fuchsin solution and then decolorized with the acid methylene blue. If but little pus is present the urine may be centrifugalized.

Using the above method Walker states that he could demonstrate tubercle bacilli in each case in which tuberculosis was afterward found.

In doubtful cases animal inoculation should be practised. The urine is received by the catheter into a sterile bottle, the first portion being allowed to escape. After twelve hours the supernatant fluid is poured off and the sediment drawn into a sterile hypodermic syringe. The material is injected into the subcutaneous tissue of the back of a guinea-pig. If tubercle bacilli are present tuberculosis should develop in from three to five weeks, but may occur even after two weeks.

Intraperitoneal injections may also be practised, although one is more apt to lose the animals from incidental infections before tuberculosis may become manifest. It is said that such secondary organisms may be eliminated by heating the material for ten minutes at 60° C. The appearances seen at autopsy are very characteristic. The spleen, lymph glands, and liver show marked lesions. In cases where death occurs rapidly (in two weeks) miliary tubercles will be seen all over the liver and spleen, while the lymph glands are only moderately enlarged. In less active cases the lymphatic picture is most pronounced; axillary, cervical, and peritoneal glands are very much enlarged and the spleen may be transformed into one huge, caseating mass.

On repeated occasions *smegma bacilli* have been mistaken for tubercle bacilli. They are quite common and especially met with in women; this, however, only in non-catheterized specimens. Greenbaum states that after thoroughly wiping the meatus and introducing a sterile catheter he never found them.

In the male confusion with the smegma bacillus is less likely to occur, and if pains are taken to wash the glans and to irrigate the urethra, as advised by Young and Churchman,[1] it may be eliminated altogether as a disturbing factor. To this end the following technique is recommended: The foreskin, if present, is rolled back and held back by the patient. The glans is thoroughly scrubbed with soap and water. This must be done with great care, using very large amounts of water for the rinsing. An irrigator is filled with sterile water and the nozzle attached. This is made from a piece of small-caliber glass tubing with a circumference of a 15 F. sound and about seven and one-half inches long. The sharp edges of one end are rounded by fusing in the Bunsen flame. The other end is inserted into a piece of rubber tubing of the proper diameter to make a snug fit. The glass tube is pushed into the rubber tube about one inch, leaving about six and a half inches free. A rubber guard (conveniently made from one-half of a rubber ball) is fitted snugly over the rubber tubing near its end, about six and one-half inches from the fore end. The nozzle is connected with the tube of an ordinary irrigator, hung high enough to give a good pressure, the patient being instructed to keep his sphincter urethræ closed during the procedure. The water is then allowed to flow, the glans and meatus well rinsed with it, and the nozzle gradually inserted and passed back to the triangular ligament (the tube is long enough to reach this), the stream flowing constantly during its insertion and withdrawal. A quart of irrigating fluid is used. (Young and Churchman.)

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[1] Amer. Jour. Med. Sci., July, 1905, p. 52.

Whether any reliable staining method exists whereby the smegma bacillus can be definitely distinguished from the tubercle bacillus seems doubtful. Trudeau suggests staining in the usual way with carbol fuchsin, to decolorize with 25 per cent. nitric acid, then to wash and place the specimens for two minutes in 95 per cent. alcohol, and to counter-stain with blue. But he states that he does not find any method reliable in all cases and in doubtful cases advises inoculation of a guinea-pig.

Of great interest is the frequent occurrence of the *typhoid bacillus* in the urine of typhoid-fever patients. Bouchard[1] in 1881 drew attention to the elimination of the bacillus through this channel, and stated that he was able to demonstrate its presence in 50 per cent. of his typhoid-fever cases. Other observers were less successful, but with improving technique and more general investigation a larger number of positive results is being obtained every year.[2] At the present time it may be said that the typhoid bacillus can be found in the urine of from 20 to 30 per cent. of all typhoid-fever patients. The organism usually appears in the second or third week of the disease, and may persist for months and even years. When present it usually occurs in pure culture, and often the bacilli are so numerous as to render cloudy a freshly voided specimen of urine. Symptoms of cystitis and marked renal involvement often occur, but in a considerable number of cases there are no indications of local disease. The elimination of the organism in the urine is of no prognostic significance, but is important from the standpoint of prophylaxis. Of special interest is the fact that the organism may at times be found in the urine, although the patient is not the subject of typhoid fever at the time. Brown[3] thus reports the case of a woman in whom a cystitis developed on the ninth day following an abdominal operation, and in whom it was thought that the typhoid bacillus was accidentally introduced by the catheter. The patient had had typhoid fever thirty-five years previously. Young[4] gives the history of a patient in whom cystitis developed during an attack of typhoid fever, owing to infection with the typhoid bacillus. The organism could still be demonstrated in the urine after several years. A double infection

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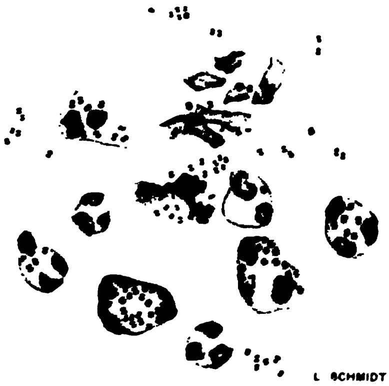
[1] Rev. de méd., 1881, p. 671.

[2] For an account of the literature, see T. R. Brown, "Cystitis due to the Typhoid Bacillus," etc., Med. Record, March 10, 1900.

[3] Loc. cit.

[4] "Chronic Cystitis due to the Bacillus Typhosus," Maryland Med. Jour., Nov., 1901, p. 456.

PLATE XXI.



L. SCHMIDT FEC.

thral Discharge from a Case of Gonorrhea, showing Gonococci Enclosed in Pus Corpuscles and Lying Free in the Discharge. Stained with Methylene Blue. (Personal Observation.)

with the gonococcus subsequently occurred, and four months later typhoid bacilli and gonococci were both present in considerable numbers. Cystoscopic examination showed a chronic ulcerative cystitis. Two additional cases of chronic cystitis due to the typhoid bacillus are reported.

The bacillus may be isolated and identified according to the usual methods. (See Blood and Feces.)

In cases of *paratyphoid* fever the corresponding bacilli may be found in the urine.

Gonococci may be found in urinary sediments enclosed in pus cells, and can be demonstrated by preparing smears and staining with a basic dye or with the eosinate of methylene-blue solution. In the so-called gonorrheal threads they can often be found years after the infection (Plate XXI).

In cases of *bubonic plague* Kitasato's coccobacillus may be found in the urine.

In cases of cystitis a great variety of microörganisms has been met with in the urine. Among the more important may be mentioned the Staphylococcus aureus, albus, and citreus, streptococci, the Bacillus coli communis, the Bacillus pyocyaneus, the Bacillus typhosus, the Proteus vulgaris, the gonococcus, etc. In many cases of cystitis organisms are found, moreover, which are apparently non-pathogenic, and are capable of causing the formation of hydrogen sulphide from certain sulphur bodies of the urine. (See Hydrothionuria.)

In conclusion, reference should be made to the occasional occurrence of a form of bacteriuria which is not associated with any pathological process, and has hence been termed *idiopathic bacteriuria.* Of its causation and significance nothing is known, but it is possible that in these cases a few bacteria enter the bladder either through the anterior rectal wall or are eliminated through the kidneys from the blood current. Finding a suitable medium for their growth in the urine, they here multiply and may thus be constantly present. Of late, the Bacillus lactis aërogenes has been found in such a case. The diagnosis "idiopathic bacteriuria" should, of course, only be made if every possible source of contamination of the urine can be definitely excluded.[1]

Urines containing bacteria in large numbers are always cloudy, and usually present an acid reaction when voided unless cystitis exists at the same time. Attention is directed to their presence by the fact that such specimens cannot be cleared by simple filtration.

Actinomyces kernels may be observed in the urine when the disease in question has attacked the genito-urinary tract or when the organism has found its way into the urine from other organs.

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[1] Roberts, "On Bacilluria," Trans. Internat. Med. Cong., London, 1881, vol. ii, p. 157. Schottelius u. Reinhold, Centralbl. f. klin. Med., 1886, vol. viii, p. 635. Ross, Baumgarten's Jahresber., 1891, vol. vi, p. 360.

Yeast cells in large numbers are usually only seen in urines containing sugar. When a chemical examination has not been made their demonstration will be of importance, as suggesting the possible existence of glucosuria.

Molds are usually seen in old diabetic urines after alcoholic fermentation has taken place, but they may also occur, though far less frequently, upon the surface of putrid urines that have contained no sugar.

The urinary *sarcina* which is at times met with is smaller than the sarcina of the gastric contents, but closely resembles it in appearance. It is of no clinical significance.

Animal Parasites.—The organism which Hassal saw in a urine that had been "freely exposed to the air" and was alkaline, and which he termed Bodo urinarius, was in all probability an infusorial monad. Salisbury was the first to point out that the *Trichomonas vaginalis* of Donné may at times occur in the bladder, but he gave no detailed account of his cases. Künstler, Marchand, Miura, and Dock subsequently reported cases in which flagellate protozoa were found, and modern research leaves no doubt that the organisms described by these observers are identical with the trichomonas of Donné. In Miura's case the habitat of the parasite was the urethra, and an examination of the patient's wife revealed the presence of similar organisms in the vagina. Künstler's case was one of pyelitis following cystotomy. Marchand's patient had a fistula in the perineum following suppuration in the pelvis, of unknown origin; cystitis did not exist. Dock's case was associated with hematuria.[1] During the past few years I have seen the same organism in several cases, 2 of which occurred in the practice of Dr. W. M. Lewis, of Baltimore. Most of them were women, and I have no doubt that the parasite found its way into the bladder from the vagina, where it could be demonstrated in 2 instances. Curiously enough a history of hematuria was obtained from 4 patients. In 2 cases the urine contained blood at the time of the examination. In 1 case there was evidence of nephritis; cystitis did not exist. The number of the parasites was variable, and sometimes quite large.

Bälz observed innumerable amebas in the turbid urine of a girl the subject of phthisis, which he described as being of larger size than the Amœba coli. Jürgens found amebas in a patient suffering with a tumor of the bladder. Wijuhoff reports their presence in the urine in 4 cases. Posner cites 1 instance and Musgrave and Clegg another, the latter a case of hemorrhagic cystitis.

In cases of bilharziasis the ova of the parasite (see Blood) are encountered in the urine together with blood. Sometimes the entire bulk of the urine is blood-tinged, but more often only the last few drops contain blood, and in these last drops the eggs of the parasite will also be found. In doubtful cases it is always best to examine this portion. The eggs are readily seen with a low power. (See Fig. 56.)

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[1] Amer. Jour. Med. Sci., January, 1896.

Filaria embryos may be found in the urine in cases of filarial chyluria. They should be looked for in the coagulum, a bit of which is teased out and pressed between two slides.

Billings and Miller[1] have reported the possible occurrence of the Anguillula aceti in the urine, in cases in which the urine is collected in bottles that had contained old vinegar. The worm very closely resembles the Anguillula stercoralis. Stiles has made a similar observation.

Echinococcus hooklets and fragments of cysts may also be found, and in rare instances ascarides find their way into the urinary passages. Bothriocephalus linguloides (Leuckart) was found in the urine in a case occurring in Eastern Asia. Eustrongylus gigas is likewise found very rarely. Moscato records one case in which chyluria existed at the same time. In Clark's case, which was reported in the United States, the passage of the worm was accompanied by hematuria.

Tumor Particles.—Tumor particles are so rarely seen in the urine that a detailed account of their occurrence may be omitted, particularly as it is seldom possible to base the diagnosis of tumor upon the presence of fragments in the urine, the clinical history and the physical signs being usually sufficient to reach a satisfactory diagnosis.

Foreign Bodies.—Of foreign bodies which may be found in the urine may be mentioned particles of fat, fibers of silk, linen, and wool, etc.; in short, material the presence of which is owing to the use of unclean vessels for the reception of the urine. Fecal matter may be passed by the urethra; such an occurrence, of course, always indicates the existence of an abnormal communication between the bowel and the urinary passages. Hair derived from a dermoid cyst may similarly be found. In hysteria foreign bodies of almost any kind, such as hair, teeth, fish-bones, wood, etc., and even snakes and frogs, may be shown the physician as having been passed in the urine. I had occasion to examine "gravel" "passed" from time to time by a hysterical patient in large amounts, "every attack being accompanied by agonizing pains shooting down into the lower abdomen;" the gravel upon examination proved to be mortar obtained from the cellar of the patient's house.

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[1] Trans. Assoc. Amer. Phys., 1902, p. 161.

CHAPTER VIII.

TRANSUDATES AND EXUDATES.

In health the so-called serous cavities of the body contain very little fluid, and quantities sufficient for analytical purposes can normally only be obtained from the pericardial sac. In pathological conditions, on the other hand, large accumulations of fluid may be observed, not only in the serous cavities, but also in the areolar connective tissue, beneath the skin, and beneath the muscles. When due to circulatory disturbances, or a hydremic condition of the blood, such accumulations of fluid are spoken of as *transudates*, while the term *exudates* is applied to similar accumulations of inflammatory origin.

Clinically, it is frequently difficult to distinguish between transudates and exudates, and large ovarian, pancreatic, and hydatid cysts, as well as cystic kidneys, may at times be mistaken for ascites. In such cases a careful chemical and microscopic examination of the fluid in question may be of value. Very frequently, moreover, it is possible *only* in this manner to determine the nature of the disease, and *the free use of the trocar and the aspirating needle in diagnosis cannot be too strongly advocated.*

TRANSUDATES.

General Characteristics.—Transudates are usually serous in character, when they present a light straw color; at times, however, owing to admixture of blood, they have a reddish tinge, and are then said to be sanguineous; in rare instances they are chylous.

Specific Gravity.—The specific gravity varies somewhat according to the origin of the fluid, but is usually lower than that of serous exudates occurring in the same cavities—one of the most important points of difference between the two kinds of fluid. Thus, in acute pleurisy the specific gravity of the exudate is usually higher than 1.020; and in chronic pleurisy, if an accumulation of pus exists at the same time, higher than 1.018, reaching even 1.030. In transudates into the pleural cavity, on the other hand, referable to circulatory disturbances, for example, as in cases of hepatic cirrhosis or cardiac insufficiency, the figures obtained are usually lower than 1.015. Transudates of peritoneal origin similarly present a specific gravity varying between 1.005 and 1.015, while that of exudates frequently reaches 1.030.

As the chemical composition, in so far as the mineral constituents and extractives are concerned, is practically the same in both classes of fluid, the difference in the specific gravity appears to be essentially due to the amount of albumin present, viz., serum albumin and serum globulin. It may be demonstrated, as a matter of fact, that exudates contain far more albumin than transudates, the amount varying between 4 and 6 per cent. in the former, as compared with 1 and 2.5 per cent. in the latter. The largest amounts of albumin in transudates are found in those of pleural origin, while in edema not more than 1 per cent. is usually present.

Reuss suggests the following formula for the purpose of determining from the specific gravity the amount of albumin in transudates and exudates:

$$E = \frac{3}{8}\,(S - 1000) - 2.8,$$

in which *E* indicates the percentage amount of albumin and *S* the specific gravity taken by means of an accurate urinometer.

Subsequent examinations have shown, however, that this formula is not applicable, since the amount of albumin is not strictly proportionate to the specific gravity.

Since the use of Esbach's albuminimeter is totally insufficient for this purpose Strubell, Reiss, Strauss and Chajes, and Engel[1] suggest a refractometric examination, which depends essentially upon the amount of albumin present, but even with this method the results are not always satisfactory. Engel lauds it, however, nevertheless. An analysis of his data follows:

	Pleura.	Abdomen.	Pericardium.
Nephritic transudates . .	1.3375	1.3374	1.3398
	1.04 per cent.	0.98 per cent.	2.29 per cent.
Cachectic transudates . .	1.3385	1.3382	1.3398
	1.59 per cent.	1.42 per cent.	2.29 per cent.
Static transudates . . .	1.3392	1.3398	1.3405
	1.97 per cent.	2.29 per cent.	2.66 per cent.
Pleuritic exudates . . .	1.3446		
	4.89 per cent.		
Peritoneal exudates . . .	. . . .	1.3445	
		4.84 per cent.	
Pericardial exudates . . .	. . . .	. . . .	1.3460
			5.64 per cent.

The upper average figures indicate the refractometric coefficient, and the figures below the corresponding amount of albumin, as calculated from Reiss' tables. For a detailed description of the method the reader is referred to Reiss' paper.[2]

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[1] Strubell. Münch. med. Wochensch., 1902, p. 616. Reiss, Arch f. exper. Pathol. and Pharmak. vol. li. Strauss and Chajes, ibid., vol. lii. Engel. Berlin. klin. Woch., 1905, p. 1364.

[2] Verhandl. d. 76 Versammlung deutscher Naturforscher u. Aerzte, Breslau, 1904.

The fact that transudates do not coagulate spontaneously in the absence of blood may further serve to distinguish them from exudates, in which a coagulum is frequently observed after standing for twenty-four hours. Not much reliance should be placed upon this point of difference, however, as exudates likewise do not always coagulate, and clotting of transudates in the presence of blood may take place within the body.

Literature.—Reuss, Deutsch. Arch. f. klin. Med., vol. xxviii, p. 317. Runeberg, ibid., 1884, vol. xxxiv, pp. 1 and 266; and Berlin. klin. Woch., 1897, No. 33. Citron, ibid., 1897, p. 854; and Deutsch. Arch. f. klin. Med., vol. xlvi. Ranke, Mittheil. a. d. med. Klin. z. Würzburg, 1886, vol. ii, p. 189.

CHEMISTRY OF TRANSUDATES.

An idea of the chemical composition of the various forms of transudates may be formed from the following tables, taken from Hoppe-Seyler and Hammarsten, the figures corresponding to 1000 parts by weight of fluid; the specimens were taken from one individual:

	Pleura.	Peritoneum.	Edema of the feet.
Water	957.59	967.68	982.17
Solids	42.41	32.32	17.83
Albumin	27.82	16.11	3.64
Ethereal extract	14.59	5.27	0.50
Alcoholic extract		10.94	3.71
Aqueous extract			1.10
Inorganic salts			9.00
Errors of analysis			0.12

Analysis of Hydrocele Fluid.

Water	938.85
Solids	61.15
Fibrin (formed)	0.59
Globulins	13.52
Serum albumin	35.94
Ethereal extract	4.02
Soluble salts	8.60
Insoluble salts	0.66
Sodium chloride	6.19
Sodium oxide	1.09

Sugar and uric acid in small amounts are also, as a rule, found in transudates, and in one case of hepatic cirrhosis Moscatelli succeeded in demonstrating the presence of allantoin. v. Jaksch states that he has frequently been able to demonstrate the presence of urobilin in both transudates and serous exudates, even though red blood corpuscles and blood-coloring matter in solution were absent. Stich also reports that in the ascitic fluid removed during life from a patient with hemorrhagic nephritis, urobilin was present. Peptone is never found; and Pajikull states that nucleo-albumin is not present in transudates of non-inflammatory origin. Hammarsten, together with Pajikull, could, however, demonstrate an albuminous substance in transudates which was regarded as a mucoid and which is present in exudates in small amounts only. It is rich in reducing substance and contains more nitrogen than the true mucins.

LITERATURE.—Moscatelli, Zeit. f. physiol. Chem., 1889, vol. xiii, p. 202. v. Jaksch, Zeit. f. Heilk., 1891, vol. xi, p. 440. Eichhorst, Zeit. f. klin. Med., 1881, vol. iii, p. 537. Stich, Münch. med. Woch., October 29, 1901.

MICROSCOPIC EXAMINATION.

Upon microscopic examination only a few isolated leukocytes and endothelial cells from the serous surfaces and undergoing fatty degeneration are usually seen. Mast-cells and eosinophilic leukocytes have been observed in the ascitic fluid in cases of myelogenous leukemia. Charcot-Leyden crystals were present at the same time. In cases in which the transudates have been confined for a long time plates of cholesterin are frequently found. They are especially abundant in hydrocele fluid. Amebas have been found by Miura in the ascitic fluid of a woman afflicted with an abdominal tumor; at the same time they were present in the stools. Leyden and Schaudin likewise met with ameboid bodies in the ascitic fluid obtained from two cases of abdominal tumor. The technique which should be employed in the microscopic examination of transudates is described below.

EXUDATES.

Exudates may be serous, serofibrinous, hemorrhagic, seropurulent, purulent, putrid, chylous, or chyloid. Of these, the seropurulent, purulent, and putrid types are manifestly of inflammatory origin, while in the case of the serous, serofibrinous, and hemorrhagic forms it may at times be difficult to determine whether the fluid represents a transudate or whether it is an exudate. A detailed chemical and microscopic examination may then be necessary.

Serous exudates are clear, of a light straw color, and present a specific gravity which usually exceeds 1.018 (1.012 to 1.024). There is a large amount of fibrin and of albumin. If blood corpuscles are present in sufficient numbers to impart a distinct red color to the fluid, it is termed *hemorrhagic;* the color may then vary from a light pink to a dark red. On standing, even the purely serous exudates generally undergo a certain degree of coagulation, which becomes more marked in the presence of blood; exceptions, however, do occur. Most important is the microscopic examination of the exudates. Generally speaking, the same methods are here employed as in the case of the blood, but the interpretation of the findings is not always easy. This is largely owing to the fact that the leukocytes often show evidence of degeneration, and that the fluid may contain endothelial cells in addition to the morphological elements of the blood, which further increases the difficulties attending a proper classification. (See Pus.) The principal point at issue in the study of the cellular elements of exudates is the question as to the predominance of either lymphocytes or the polynuclear elements of the blood. Widal and his collaborators, more especially, have pointed out that whereas in exudates of non-tuberculous, acute inflammatory origin the polynuclear neutrophilic leukocytes predominate, the lymphocytes prevail in the chronic tuberculous forms. His observations have, on the whole, been confirmed by numerous investigators, and the importance of *cytodiagnosis* in pleuritic effusions more especially is now well established. From the available data we may formulate the following conclusions: In the very earliest stages of tuberculosis involving the serous membranes there is found a variable number of neutrophilic leukocytes in addition to lymphocytes and endothelial cells. Very soon, however, they diminish and in the later stages the lymphocyte is by far the predominating cell, while the neutrophilic elements are present only in very small numbers. Generally speaking the percentage of lymphocytes in tuberculous pleurisies ranges from 50 to 98, increasing as the disease continues.

In pleuritic effusions due to the pneumococcus and to streptococci during the serous stages, the neutrophilic leukocytes far outnumber the lymphocytes. (Average in postpneumonic cases 71.7: variations from 58 to 92.5 per cent.)[1] In the pneumococcus cases, moreover, it is common to meet with large numbers of endothelial cells, sometimes containing polynuclear leukocytes and red cells in their interior.

In cases of traumatic and aseptic pleurisy, in association with diseases of the heart and kidneys, large endothelial cells are seen which often present most grotesque appearances, occurring either singly or in groups of two, three, four or more; while the occurrence of large numbers of such cells has been regarded as characteristic of transudates, Carter has shown that in these cases also there may be a lymphocytosis of from 86 to 100 per cent.; so that confusion may arise in differentiating these cases from tuberculous pleurisy. The low specific gravity—average about 1.008—and the small amount of fibrin and albumin in the transudates will, however, aid in arriving at a conclusion.

French writers also describe a pleural eosinophilia in which large numbers of eosinophilic cells—6 to 54 per cent.—are found in the effusion, while in the circulating blood their number is not increased. Ravaut reports 4 cases of this kind. In 1 the effusion occurred secondarily in the course of syphilis; in the second in a case of typhoid ever; the third was a case of phthisis, while in the fourth no diagnosis as made. I have recently seen a case of this kind (probably tuber- ulous) with 10 per cent. of eosinophiles, 4 per cent. neutrophiles, 83 er cent. of small mononuclears, and 3.4 per cent. of large mononu- lears in the exudate, and 3.5 per cent. of eosinophiles, 42 per cent. of eutrophiles, 36 per cent. of small mononuclears, and 18 per cent. of arge mononuclears in the blood.

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[1] H. S. Carter, Med. News, October 1, 1904

Carter[1] reports 2 cases of pleural effusion, referable to pistol-shot ounds of the chest walls, in which the eosinophiles numbered 70.2 nd 87.8 per cent., respectively.

Mast-cells are rarely seen in pleuritic effusions, and it has been bserved that their granules are then quite readily soluble in water, o that they cannot be demonstrated with aqueous solutions of the sual dyes. Wolff notes a case in which the mast-cells constituted bout 10 per cent. of the total number of leukocytes.

Whether or not the conclusions which have been reached regard- ng the meaning of the prevalence of certain cell forms in pleural ffusions can be directly applied in the case of ascitic fluid remains o be seen. From the available data it appears that the indications are ot as direct. But generally speaking endothelial plaques control the icture in ascites of mechanical origin, while lymphocytes predomi- ate in tuberculous peritonitis and in peritoneal carcinoma. The ccurrence of large vacuolated cells is suggestive of a cyst accompanied y ascites (ovarian cyst). The same is true of the cytological study of oint effusions. Widal reports that in 3 cases of acute rheumatism e found polynuclear leukocytes in the serous exudate, while they ere absent in traumatic cases of arthritis. As the result of an xamination of 30 hydroceles Marchetti[2] concludes that lymphocyte nd epithelial cells predominate without exception.

Of the cytological findings in the cerebrospinal fluid a detailed ccount will be given later.

Generally speaking the cytological factor does not seem to depend o much upon the anatomical localization of the morbid process as pon its duration and the character of the pathogenic agent. An cute process (pneumococci, streptococci) call forth a lymphocytosis f brief duration, which is followed sooner or later by a granulocytosis, hile a less intense stimulus, and one acting more slowly (tubercle acillus) leads to a persistent lymphocytosis. The possibility that a timulus of the latter order may act with undue virulence and ntensity, and that one of the first type may be exceptionally mild nd delay the occurrence of granulocytosis, should, however, be orne in mind.

Very important also is the study of the cellular elements which are found in serous exudates in cases of malignant disease of the serous membranes. Difficulty may here be encountered in the interpretation of the cellular findings, for on the one hand it is often difficult to distinguish the endothelial cells from leukocytes, as they take on phagocytic activity and often present the most bizarre forms. The nucleus, which is normally centrally located, takes up an excentric position, and enclosed within the cell we may find leukocytes and red cells. On the other hand, it is impossible by simple inspection to distinguish normal endothelial cells from *cancer cells*. In cases of doubt it is well to ascertain whether the epithelial elements give the glycogen reaction and to hunt for the presence of mitosis. Qunicke has pointed out that normal endothelial cells do not contain glycogen, and that a marked iodine reaction is very suggestive of carcinoma. Wolff, however, suggests that this test is probably not specific, and cites two instances in which he obtained a positive glycogen reaction, although a tumor did not exist. More important probably is the presence of mitoses. In non-malignant exudates epithelial cells never present evidence of mitosis, while in cases of tumor they may be found. Rieder regards their occurrence as pathognomonic of malignant disease. Commonly the mitosis is atypical; the division of the nucleus is not followed by a division of the cell; the chromosomes are short and show no polar or equatorial arrangement.

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[1] Med. News, October 1, 1904.

[2] Gaz. d. Ospedal. e. d. clin., 1904, No. 94.

In cases of neoplasm Quincke has also drawn attention to the occurrence of large numbers of fat droplets in the fluid, which may attain a diameter of from 40 to 50 μ. At times, however, the fat droplets are so small and so numerous as to give a chylous appearance to the exudate. At other times a similar appearance is due to the presence of minute albuminous granules, which may be distinguished from fat by their insolubility in ether and the fact that they are not stained with the common fat dyes, such as Sudan, scarlet-R, and alkanin. The occurrence of numerous fatty acid crystals, arranged in groups, should also excite suspicion of a neoplasm.

Should bits of tissue be obtained, a positive diagnosis of malignant disease may, of course, be made by the usual methods. Such particles should be placed at once in absolute alcohol or formalin.

Crystalline elements are not usually seen in serous or hemorrhagic exudates; at times we meet with platelets of cholesterin.

Technique.—In every case the fluid should be examined as soon after puncture as possible; if this cannot be done at once, coagulation may be prevented by the addition of sodium citrate. The material is then placed in the ice-box until a sediment has collected or this may be obtained at once by centrifugation, new portions of fluid being repeatedly used and the sediments combined. Cover-glass preparations may then be conveniently made, or smears on slides exactly as in the case of blood, care being taken to do as little injury to the cellular elements as possible. The smears should be very thin, so that the specimens will dry rapidly and but little chance is given for the cells to contract beyond their usual size. Subsequent treatment will depend upon the special points which are to be elicited. Unfortunately the leukocytes are often much changed, so that their classification may be attended by considerable difficulties. The polynuclear elements may appear mononuclear and not infrequently the neutrophilic granules can no longer be demonstrated. (See Pus.) For this reason the triacid stain is not to be recommended for routine work; the eosinate is much better and will furnish as satisfactory results as can be obtained with a panoptic dye. Successive staining with eosin and methylene blue sometimes gives better results than a polychrome dye. Care should be had not to diagnosticate eosinophilia from the fact that cell granules are stained red, as the neutrophilic granules of degenerating cells are commonly amphophilic, viz., they stain both with acid and neutral dyes; account must be taken of the size of the granules and the general structure of the cell. To differentiate pseudolymphocytes from true lymphocytes, Pappenheim's methyl-green pyronin may be employed, though it is not absolutely specific; still it will be found that even though the protoplasm of other cellular elements may take the red color of the pyronin, the intensity is distinctly less than in the case of the lymphocytes proper.

Pappenheim's Method.[1]—The stain is composed of a concentrated aqueous solution of methyl green to which pyronin is added until the solution just begins to turn blue viz., about 1 part of pyronin for 3 to 4 parts of methyl green. Stained in this manner the basophilic protoplasm of the lymphocytes is colored a fine dark carmine red, while the protoplasm of all other cells is stained a more or less pale brownish or reddish yellow, or remains colorless. Pappenheim regards this stain as essentially specific for the lymphocytes, but admits that it also stains in a similar manner the young erythroblasts that are poor in hemoglobin. The difference can be recognized from the character of the nuclei and the fact that the margin of the lymphocytes very commonly appears shaggy, while that of the erythroblasts is smooth and homogeneous.

To study *mitosis*, hematoxylin and eosin may be employed, or the Romanowsky method in one of its various modifications.

The glycogen reaction is demonstrated as in the case of the blood

BACTERIOLOGICAL EXAMINATION OF EXUDATES.

In a measure the bacteriological examination of exudates has been supplanted by the cytological study, as outlined above; especially as the bacteriological examination has been notoriously unsatisfactory in the most important group of effusions, viz., in those of tuberculous origin. It is now known that *all* exudates gradually become free from bacteria, even though at first they may have been caused by bacterial activity. As a result it is no longer justifiable to conclude that a process is tuberculous because bacteriological examination of the exudate has given no positive result. If it is desired to cultivate organisms that may be present, it is well to make a bouillon culture in every case so as to eliminate the bactericidal properties of the exudate as much as possible. In any event it is well to centrifugate the fluid in a sterile tube and to use the sediment for inoculations. The organisms which are most likely to be encountered are the pneumococcus, the various staphylococci, streptococci, and more rarely the colon bacillus and the typhoid bacillus.

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[1] Virchow's Archiv, 1899, vol. clvii.

Inoscopy.[1]—Jousset recommends the following procedure for the purpose of demonstrating tubercle bacilli in exudates: The fluid is allowed to clot spontaneously or by adding a little horse serum. The clot, which is supposed to contain most of the organisms, is pressed out, torn into fragments, and placed in about 10 c.c. of a digestive mixture of the following composition: pepsin, 1 to 2 grams; glycerin, 10 c.c.; 40 per cent. solution of hydrochloric acid, 15 c.c.; sodium fluoride, 3 grams; water, 1000 c.c. The material is left in the incubator for three to four hours, then centrifugalized and smears prepared from the sediment and stained as usual. Jousset claims to have obtained very good results in this manner, while others are less enthusiastic.

More recently Zebrowski[2] has suggested the following method as more likely to lead to satisfactory results: Coagulation of the fluid is prevented by the addition of an equal volume of a 0.5 per cent. solution of sodium fluoride. The mixture is set aside in a cool place until the following day, when it is thoroughly centrifugated and smears made from the sediment and stained as usual.

With this method Zebrowski claims to have found tubercle bacilli in 83 per cent. of secondary and 55 per cent of primary pleurisies.

More satisfactory than either method possibly is the animal experiment, to which end a large quantity of the fluid is centrifugalized and the sediment injected into the peritoneal cavity of a guinea-pig, as in the case of the urine (which see).

LITERATURE.—Widal and Ravaut, "Cytodiagnostique des epanchements serofibrineux de la plèvre," Trans. XIII Internat. Med. Cong. Paris, 1900. Barjou and Cade, "Etudes cytol.," etc., Arch. gén. d. méd., August, 1902. Gulland. "Cytodiagnosis," etc., Scott. Med. and Surg. Jour., June, 1902, p. 490. A. Wolff, "Transudates and Exudates," Zeit. f. klin. Med., 1902, vol. xxii, Heft 5 u. 6. Quincke, Deutsch. Arch. f. klin. Med., 1882, vol. xxx, pp. 369 and 580. Rieder, ibid., 1895, vol. liv, p. 544.

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[1] La semaine méd., 1903, No. 3.

[2] Deutsch. med. Woch., September 7, 1905.

CHEMISTRY OF EXUDATES.

According to Moritz, an albumin is found in exudates that can be precipitated with acetic acid and which is absent in transudates. He regards this as serum globulin which has undergone a change as a result of the inflammatory process. According to Matsumoto, on the other hand, the substance in question represents a mixture of fibrinoglobulin, euglobulin, and a small amount of pseudoglobulin; in the filtrate, however, there is also some fibrinoglobulin (fibrinogen) and euglobulin. He suggests that this last circumstance is probably referable to the small amount of salt in exudates and that in the first instance the pseudoglobulin is probably carried down mechanically.

More recently Umber has studied the body in question and arrived at the conclusion that it belongs to the mucins. To its presence the mucinous character of such fluids is due. It is precipitated by the addition of acetic acid and is insoluble in an excess of the reagent unless the acid is present in great concentration. The body has markedly acid properties and is not coagulated by heat. It differs from the known mucins in the presence of a very small amount of reducing substance, which can only be demonstrated by special methods. It contains about 14 per cent. of nitrogen and no phosphorus. In neutral and feebly acid solution the substance does not coagulate (thus differing from the globulins). The same body apparently was found by Salkowski in an exudate into the hip-joint. Umber calls this substance *serosamucin.* Its amount is less than 0.5 per cent.

According to Umber and Stähelin the serosamucin is essentially found in exudates referable to inflammatory processes or associated with new growths. In transudates, as Runeberg already pointed out, only a very slight turbidity results upon the addition of acetic acid, and not in all cases, moreover; so that a well-marked reaction, viz., a marked precipitation upon the addition of acetic acid to the point of a distinctly acid reaction, may be regarded as a valuable sign in the diagnosis between transudates and exudates. I append some of the results obtained by Umber:

ASCITES.

	No. of cases	Serosamucin
Hepatic cirrhosis	6	0
Hepatic cirrhosis with chronic nephritis and phthisis	1	0
Nephritis	1	0
Mitral disease	2	0

PLEURAL EFFUSIONS.

	No. of cases	Serosamucin
Degeneratio cordis and nephritis	2	0
Myocarditis	1	0
Hepatic cirrhosis	1	0
Lymphosarcoma [illegible]	1	0
Carcinoma mammae with pleural metastases	1	+
Tuberculosis of pleura	[illegible]	+
Pleuritis exudativa acuta	[illegible]	+
Pleuritis and pericarditis	1	+

For the isolation of serosamucin see Umber's paper (see Literature below.)

Of the common albumins we meet with traces of fibrinogen and with fairly large amounts of globulin and serum albumin. Their percentage may at times not appear so very large, but considering the large amount of fluid and the rapidity with which it may accumulate it is clear that the loss of nitrogen to the body in this form may be very considerable. Umber showed that in one of his cases 5000 grams of albumin representing about 15,000 grams of muscle tissue were lost within a year.

In addition to the serosamucin and the common albumins mentioned, some exudates may possibly also contain small amounts of a a nucleo-albumin, as is suggested by the findings of Pajikull. Should ovarian cysts have ruptured into the peritoneal cavity, we may further find both pseudomucin and paramucin (which see).

Of interest further is the fact that Umber succeeded in demonstrating the existence of autolytic processes in exudates. He found both albumoses and mono-amino acids, viz., leucin and tyrosin.

Coriat has reported a case of polyneuritic delirium, in which pleurisy with effusion developed. In the effusion he could demonstrate a peculiar albuminous substance, which he regards as identical with Bence Jones' albumin; in the urine this substance could not be found.

LITERATURE.—Pajikull (Swedish ref. by Hammarsten: Jahresber. f. Thierchem., 1893). Moritz, Münch. med. Woch., 1902, No. 42. Matsumoto, Deutsch. Arch., 1902, vol. lxxv, p. 409. Stähelin, Münch. med. Woch., 1902, No. 34. F. Umber, Zeitsch. f. klin. Med., 1903, vol. xlviii, p. 364. Coriat, "The Occurrence of the Bence Jones Albumin in a Pleuritic Effusion," Amer. Jour. Med. Sci., 1903, vol. cxxvi, p. 631.

Pus.

General Characteristics of Pus.—If pus, which usually presents a color varying from yellowish gray to greenish yellow, is allowed to stand for a time, a liquid gradually appears at the top, and increases in amount until it is finally possible to distinguish two distinct layers, the one above—the pus serum; the other at the bottom—the pus corpuscles. Upon the number of the latter the consistence as well as the specific gravity of the pus is dependent. This may vary between 1.020 and 1.040, with an average of 1.031 to 1.033. Fresh pus has always an alkaline reaction, which may become neutral or slightly acid upon standing, owing to the development of free fatty acids, glycerin-phosphoric acid, and lactic acid. The color of pus serum may be a light straw, a greenish or a brownish yellow.

Chemistry of Pus.—The chemical composition of pus serum and pus corpuscles may be seen from the following tables:

Analysis of Pus Serum.

	I.	II.
Water	913.70	905.65
Solids	86.30	94.35
Albumins	63.23	77.21
Lecithin	1.50	0.56
Fat	0.26	0.29
Cholesterin	0.53	0.87
Alcoholic extract	1.52	0.73
Aqueous extract	11.53	6.92
Inorganic salts	7.73	7.77

Analysis of Pus Corpuscles.

	I.	II.
Nuclein	342.37	673.69
Insoluble matter	205.66	
Albumins	137.62	
Lecithin	143.83	75.64
Fat		75.00
Cholesterin	74.00	72.83
Cerebrin	51.99	102.84
Extractives	44.33	

Albumoses are usually present, and are derived from the pus corpuscles. Leucin and tyrosin are likewise frequently met with in the pus of old abscesses; and fatty acids, urea, sugar, glycogen, biliary pigments and acids (in catarrhal jaundice), acetone, uric acid, xanthin bases, cholesterin, etc., have occasionally been observed.[1]

Microscopic Examination of Pus. Leukocytes.—If a drop of pus is examined with the microscope, it will be seen to contain innumerable leukocytes, many of which in perfectly fresh pus exhibit ameboid movements. The cells in question are usually almost altogether of the neutrophilic variety, and it may be questioned whether the lymphocytes ever occur in true pus. Even in cases of lymphatic leukemia the predominating cell in abscesses is the polynuclear leukocyte or its degeneration forms. Mononuclear elements with basophilic protoplasm, however, are also met with, notably in the more chronic cases, but it is likely that they are derived from the connective-tissue cells and are not of hematogenic origin. Eosinophiles are only seen in pus under certain definite conditions, as in gonorrhea (see below), and mast-cells also are quite uncommon.

In pus that is not perfectly fresh it is usually not possible to demonstrate the presence of neutrophilic granules. In such cells, moreover, we commonly meet with fragmentation of the nucleus, associated with marked pyknosis. This was first noted by Ehrlich in a case of hemorrhagic smallpox and in various exudates, and has subsequently been described by Michaelis and Wolff. The degeneration may proceed to fragmentation of the entire cell with the consequent formation of mononuclear neutrophilic forms (Ehrlich's pseudolymphocytes). On the other hand, a type of degeneration is seen in which the nucleus does not become pyknotic, but swells to a large size and stains rather faintly with basic dyes. In such cells the protoplasm appears as a narrow rim and the impression is gained as though the cell were in reality a leukocyte; if at the same time the granules have been lost, the differentiation may indeed be impossible, unless transition forms exist between the normal polynuclear neutrophile and the type in question.[1]

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[1] M. Pickardt, "Z. Kenntniss d. Chemie path. Ergüsse," Berlin. klin. Woch., 1897, p. 844.

Owing to resorption of water from accumulations of pus of long standing, such material finally assumes a caseous aspect, and the leukocytes will be seen to have greatly diminished in size, and to have assumed an angular, shrunken appearance; it is then hardly possible to demonstrate the presence of a nucleus, even after the addition of acetic acid.

It is noteworthy that in cases of hepatic abscess referable to Amœba coli it is seldom possible to demonstrate any normal leukocytes, and it will be seen that under such conditions the pus consists almost altogether of granular and fatty detritus, while in liver abscesses due to other causes the leukocytes usually present a fairly normal appearance.

Mast-cells are only exceptionally seen in pus.

Giant Corpuscles.—So-called giant pus corpuscles, measuring at times from 30 μ to 40 μ in diameter, have been observed in abscesses of the gum, hypopyon, and in the contents of suppurating ovarian cysts, but they do not appear to have any special significance. Upon careful examination these bodies will be seen to contain one oval nucleus, usually located eccentrically within the cell, and from one to thirty or even forty pus corpuscles.[2]

Detritus.—Fatty and albuminous detritus in variable amount may be observed in every specimen of pus, and increases with the length of time that it has been confined within the body. The same holds good for the presence of free nuclei, which were formerly regarded as young pus corpuscles, but which have now been definitely recognized as originating during the disintegration of the corpuscles.

Red Corpuscles.—Red blood corpuscles in variable numbers are usually seen in every specimen, their appearance depending upon the length of time they have been confined. Pus corpuscles may at times contain a red corpuscle.

Pathogenic Vegetable Parasites.—Of the pathogenic organisms which are of especial interest from a clinical standpoint may be mentioned the true pus organisms, notably the staphylococci and the Streptococcus pyogenes; furthermore, the tubercle bacillus, the Actinomyces hominis, the bacillus of glanders, the bacillus of anthrax, leprosy, tetanus, influenza, and Fränkel's pneumococcus, etc. The majority of these have already been described. A pathogenic leptothrix, named by Flexner the L. asteroides, has been found by Cozzolino[1] in the pus of a retroperitoneal abscess.

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[1] L. Michaelis and A. Wolff, "Die Lymphocyten," Deutsch. med. Woch., 1901, vol. xxvii, p. 651.

[2] Böttcher, Virchow's Archiv, 1867, vol. xxxix, p. 512. Bizzozero, loc. cit.

A form of streptothrix has been isolated from the pus of certain cases of mycetoma, or Madura foot.[2]

Vincent's[3] fusiform bacilli and spirilla have been encountered in the pus of alveolar pyorrhœa, in noma, hospital gangrene, gangrenous ulcer of the penis, in bronchiectasis, abscess of the leg, etc.

In the pus of abscesses in cases of systemic blastomyces infection the corresponding organism is found.

Protozoa, with the exception of the Amœba coli, have only rarely been found. Künstler and Pitres[4] observed numerous large spores with from ten to twenty crescentic corpuscles in pus taken from the pleural cavity of a man, which closely resembled the coccidia of mice. Litten[5] observed cercomonads in the fluid withdrawn from a pleural cavity. Trichomonads have been found in empyema in connection with pulmonary gangrene.

Most important in this connection is the demonstration of the Amœba coli in the pus, and in cases of liver abscess an examination with this end in view should never be neglected. So far as the occurrence of amebas in *pus* is concerned, the observation of Kartulis and of Flexner, who demonstrated their presence in an abscess of the lower jaw, shows that they should not be looked for in the pus of abscesses of the liver or lung only.

In smears obtained from two cases of oriental boil (tropical ulcer, Delhi boil, Aleppo boil) Marzinowsky and Bargow,[6] on the one hand, and Wright[7] on the other, found little bodies, measuring from 1 to 4 μ in diameter and apparently provided with a macronucleus and a micronucleus. They are inclined to look upon these as protozoa and as parasitic. Marzinowsky and Bragow name the organism Booplasma orientale; Wright calls it the Helcosoma tropicum. According to Christofers[8] they are identical with the Leishmania-donovani of tropical splenomegaly, which latter are known to occur in the skin ulcers of kala-azar.

Vermes.—Of these, the filaria and hydatids are rarely observed in this country. Bothriocephalus linguloides has been found in the pleural cavity of a Chinese patient.

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[1] Zeitsch. f. Hygiene, 1900, vol. xxxiii, p. 36.

[2] Boyce and Adams, Jour. Exper. Med., vol. iii, p. 422.

[3] Annal. de l'Institut Pasteur, 1894, vol. viii, p. 129.

[4] Compt.-rend. de la Soc. de biol., 1884, p. 523.

[5] Verhandl. d. Cong. f. inn. Med., 1886, vol. v, p. 417.

[6] Virchow's Archiv, 1904, p. 178.

[7] Jour. Cut. Dis., incl. Syph., New York, June, 1904, and Jour. Med. Research, December, 1903.

[8] "Discussion on the Leishman-Donovan Body," Brit. Med. Jour., September 17, 1904.

Crystals.—As has been stated, crystals of cholesterin are frequently found in old pus and in exudates of long standing, but are rarely seen in recent exudates. They may be recognized by their characteristic form and their chemical reactions, as described in the chapter on the Feces. Triple phosphates, fatty acid crystals, and hematoidin are likewise frequently seen, the presence of the latter, of course, indicating a previous admixture of blood.

The **technique** to be employed in the examination of pus is as a rule simple. Cover-glass preparations or smears on slides are prepared as in the case of the blood and are then stained according to the points that are to be elicited. For routine work the eosinate of methylene blue will be found very useful. If the pus corpuscles are still fairly fresh, the neutrophilic granules are readily stained; it will be noted, however, that very commonly they exhibit a more decided red, which is referable to certain degenerative changes which cause the granules to assume an affinity for acid dyes as well. Bacteria that may be present are usually well shown. If the pus is older and the cells have lost their granules, Pappenheim's pyronin-methyl green will be found of value in the study of the mononuclear forms.

Gonorrheal Pus.

In the very earliest stages of the disease the pus contains large numbers of eosinophilic cells besides the common polynuclear neutrophiles.[1] But at the same time and throughout the course of the disease mononuclear non-granular elements, with basophilic protoplasm, are also seen. The larger number of the latter are of the type of the large mononuclear leukocyte and transition form of Ehrlich, but a certain percentage is also represented by the lymphocytes, both of the small and large variety. Mast-cells may also occur in gonorrheal pus; a remarkable case is reported by Neisser, in which the pus consisted practically exclusively of such elements.

The neutrophilic elements in gonorrheal pus commonly present evidence of degeneration. In some a loss of granular material has manifestly taken place, and it can be demonstrated that in most of the cells the granules are no longer absolutely neutrophilic, but have become amphophilic—that is, from a neutral mixture they take up the neutral dye, but they can also be stained with acid dyes. With the triglycerin mixture, for example, they are stained red by the eosin.

As regards the distribution of gonococci in the different cellular elements, it is noteworthy that they are principally found in the polynuclear neutrophiles, while they are less commonly seen in the mononuclear leukocytes and transition forms. In the small lymphocytes they are not encountered, and it is uncommon to find them in the eosinophilic cells.

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[1] Other observers do not mention the early occurrence of eosinophiles in the pus. Esserteau states that they are increased from the second to the fourth week.

Generally speaking numerous gonococci, eosinophiles, and a small number of lymphocytes are found in cases of recent gonorrhea, while during exacerbations of chronic processes only a few cocci and numerous mononuclear elements are encountered. The common neutrophilic elements of course control the picture practically at all times, so long as there is a discharge.

The **gonococcus** (Neisser) (Plate XXI) occurs in the form of small oval or coffee-bean-shaped granules, grouped in twos and fours resembling a German biscuit; the individual cocci measure about 1.25 μ in length by 0.7 μ in diameter. As a rule they are found enclosed within pus corpuscles and epithelial cells; but they may also occur free in the pus obtained from the urethra, in the vaginal discharge, and more rarely in urinary sediments, as in cases of complicating prostatitis, peri-urethritis, etc. In cover-glass specimens account should be taken only of those organisms which are enclosed within cellular elements, as these alone may be regarded as characteristic. To this end a drop of the discharge is spread in a thin layer upon a slide or a glover-glass, dried in the air, and fixed by passing three or four times through the flame of a Bunsen burner. The specimens may then be stained with any one of the basic aniline dyes. In my laboratory the eosinate of methylene blue is almost exclusively used for this purpose. The organisms are thus colored blue, while the granules of eosinophilic leukocytes, which may be present at the same time, appear a bright red or a brownish red. After five minutes the excess of stain is washed off, the preparations are rinsed in water, dried with filter paper, and examined with a high power.

The gonococcus is decolorized by Gram's method and can in this manner be distinguished from certain other organisms that may be present. Of the four kinds of diplococci which may be found in urethritis besides the gonococcus, only two forms are similarly decolorized, and these two are rarely seen. We may conclude that in 95 per cent. of all cases Gram's method permits a definite conclusion as to the presence or absence of the true organism. *Gram's method is* best employed in the modification suggested by Weinrich: The preparations are fixed by drawing through the flame of a Bunsen burner and are then stained for from one to two minutes in Fränkel's carbol-gentian-violet solution (10 parts of a saturated alcoholic solution of gentian violet to 90 parts of a 2.5 per cent. solution of carbolic acid). Without washing they are placed for one to three minutes in Lugol's solution (1 gram of iodine, 2 grams of potassium iodide, and 300 c.c. of distilled water), and again without washing in absolute alcohol, until the alcohol ceases to extract color (about one and one-half minutes); they are now washed in water, counterstained with Bismarck brown, washed, dried, and mounted. The Bismarck-brown solution is prepared as follows: 3 grams of the dye are dissolved in 70 c.c. of hot water; 30 c.c. of 96 per cent. alcohol are added; the mixture is well stirred and filtered.

The organism grows best on blood and hydrocele agar. The surface colonies are pale, grayish, translucent, and finely granular, with finely notched borders. In bouillon and blood serum mixed it forms a membrane, while the fluid remains clear.

When no discharge can be obtained from the urethra, or an examination of such discharge is negative, positive results may at times still be obtained if some of the *gonorrheal threads* are examined, which may be found floating in the urine. In these the organisms can occasionally be demonstrated after months and even years have elapsed after primary infection.

LITERATURE.—Janowski, Arch. f. exper. Pathol., 1895, vol. xxxvi, p. 15. L. Michaelis and A. Wolff, "Die Lymphocyten," Deutsch. med. Woch., 1901, vol. xxvii, p. 651. A. Pappenheim, Virchow's Archiv, 1901, vol. clxix, p. 72. Neisser, Centralbl. f. d. med. Wiss., 1879, vol. xvii, p. 497. J. Plato, "Ueber Gonokokkenfärbung mit Neutralroth," etc., Berlin. klin. Woch., 1899, p. 1085. E. R. Owings, "The Infectiousness of Chronic Urethritis," Bull. Johns Hopkins Hosp., 1897, p. 210. H. H. Young, "Welch Festschrift," Johns Hopkins Press, 1900, p. 677.

Putrid Exudates.

Putrid exudates are observed following perforation of a gangrenous focus or of a gastric or intestinal ulcer into one of the body cavities. At other times they are encountered in cases of neoplasm, and at times even without apparent cause. The material obtained in such cases has a brown or brownish-green color, and emits an odor which in itself indicates the character of the exudate. Microscopically, cholesterin, hematoidin, and fatty acid crystals, as well as degenerating leukocytes, are found. In cases in which aspiration of a higher intercostal space reveals the presence of serous fluid, while putrid material is obtained at a lower point, the existence of a subphrenic abscess should be suspected. In such cases a pure culture of the Bacillus coli communis has been obtained. The reaction of putrid exudates is usually alkaline, but an acid reaction may be obtained in cases of perforation of a gastric ulcer; the Sarcina ventriculi and saccharomyces may then also be found.

Chylous and Chyloid Exudates.

Chylous and chyloid exudates have been repeatedly observed. They are most frequently met with in the abdominal cavity (one hundred and four times out of a total number of one hundred and fifty-five, which have thus far been reported), less commonly in the pleural cavity (forty-nine times), and only rarely in the pericardial sac (twice only) (1904). Among the causes which may lead to chylous ascites the following are recognized (in the order of their frequency): compression of the thoracic duct or the lymphatic vessels by glandular enlargements, neoplasms, etc.; non-tuberculous peritonitis; occlusion of the left subclavian; excessive pressure, strain, cough; peritoneal carcinoma; filariasis; occlusion of the thoracic duct; occlusion of lymph vessels, external pressure; diseases of the liver, syphilis, primary disease of the lymph vessels, angioma, calculus of the receptaculum chyli, and Hodgkin's disease. Quincke believes that the two forms can be etiologically distinguished from one another by means of a microscopic examination, as the cloudy appearance in the chyloid form is usually referable to the presence of endothelial or epithelioid cells undergoing fatty degeneration. Later observations, however, have shown that the differentiation of the two forms cannot be made upon this basis, as the same anatomical lesion, such as carcinoma or tuberculosis, may at times give rise to the formation of a chylous exudate, at others to that of the chyloid form, and both, moreover, may coexist. An instance of this kind is described by Wilson.

Senator claimed that the presence of more than traces of sugar is strongly suggestive of the chylous nature of the exudate. Possibly this observation may be of some value, but only the presence of more than 0.2 per cent. is of value. Of greater significance is the fact that in chylous fluid the melting point of the fat will depend upon the melting point of the fat which was taken in as food, while this is not the case in chyloid effusions. The amount of fat, moreover, which is present is influenced directly by the amount ingested in the first instance.

Occasionally one can get the distinct odor of the food which has been taken, in chylous exudates, while in the chyloid type this would hardly be expected.

Chylous exudates in their general appearance resemble milk, while chyloid fluid is more suggestive of pus. The turbidity in both cases is usually referable to the presence of innumerable fat globules, which are especially abundant in the chylous form. In chyloid exudates the origin of the fat from cellular elements is often apparent at once; but, as has been said, it is impossible to draw definite etiological conclusions from that difference. Some chyloid exudates contain no fat at all, and Lion has shown that the milky appearance in such cases is owing to the presence of a curious albuminous substance, belonging to the class of nucleo-albumins. Bernert, on the other hand, claims that the substance in question belongs to the globulins, and is closely associated with certain lecithins. A similar observation is recorded by Micheli and Mattirolo.

Edsall (cited by Wilson) reported an instance of non-fatty pleural effusion, the opacity of which was due to altered globulins.

Chemical analysis of a chylous exudate (pleural) from a case of Hodgkin's disease, which Campbell made in my laboratory, showed the following result:

Water	90.84 per cent.
Solids	9.15 " "
Mineral solids	0.76 " "
Organic solids	8.39 " "
Coagulable albumins	4.80 " "
Fats	3.0 " "
Sugar	0.59 " "

The specific gravity was 1.020.

The cytological formula in such exudates has as yet received but little attention. In Campbell's case only a small number of leukocytes was present and most of these were of the lymphocytic type. In Muttermilch's case lymphocytes were said to preponderate; in addition there were small numbers of neutrophilic leukocytes, containing fat granules, together with eosinophilic cells and a very few red cells. In the mixed case of Wilson the lymphocytes numbered 76 per cent., and the large mononuclear cells 22 per cent.

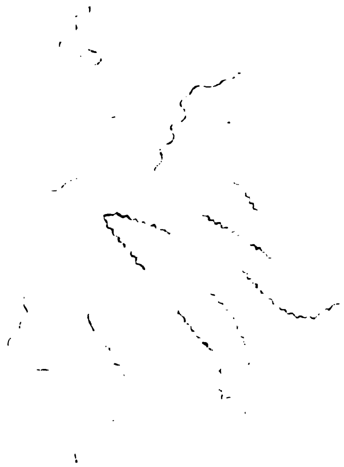
Literature.—Quincke, loc. cit. Boulengier, Schmidt's Jahrb., 1890, vol. ccxxvi, p. 28. Wilson, Amer. Jour. Med. Sci., October, 1905. Boston, Jour. Amer. Med. Assoc., February 18, 1905. Micheli and Mattirolo, Wien. klin. Woch., 1900, No. 3. Muttermilch, Zeit. f. klin. Med., vol. xlvi, p. 123. Shaw, Jour. Pathol. and Bacter., vol. vi, 1900.

Examination of Syphilitic Material.

Spirochæte Pallida.—Through the researches of Schaudinn and Hoffmann it has been ascertained that in primary and secondary syphilitic lesions a spirochæte can be demonstrated which probably represents the cause of the disease. Their results have been abundantly verified both abroad and in the United States. The organism has been demonstrated in the scrapings obtained from chancres, incised papules and condylomata, and in smears from mucous patches and the aspirated juice of the inguinal glands. Schaudinn and Hoffmann could further demonstrate the organism in the blood obtained by puncture of the spleen in a recent case of syphilis on the day preceding the eruption. Levaditi found it in the vesicular contents of pemphigus syphiliticus. Buschke and Fischer, Babes and Panea, and Levaditi found the spirochæte in the internal organs of children which had died of congenital syphilis, as also in the blood, and Metschnikoff could demonstrate it in the lesions of artificial syphilis in the ape.

The Spirochæte pallida derives its name from its low refractive power and the difficulty with which it takes up aniline dyes (this

PLATE XXII



Spirochætæ.

[illegible] refringens, S. pallida. Stained with Giemsa's [illegible]

especially in contradistinction to the Spirochæte refringens). It is a very delicate structure, usually presenting 10 to 40 deep spiral incurvations with the larger specimens, or only 2 to 4 in the smaller ones. The length varies from 4 to 10 μ with 7 μ as an average; the width does not exceed 0.5 μ. In the wet preparation it may be observed that its movements occur in an oscillatory manner about the longitudinal axis, and that, in contradistinction to the spirilla, the movements of the spirochæte are winding, bending, and whipping, while in the spirilla the longitudinal axis remains rigid. Schaudinn also demonstrated the existence of a flagellum at each end, while the other spirochetas have an undulating membrane. (See Plate XXII and Fig. 171).

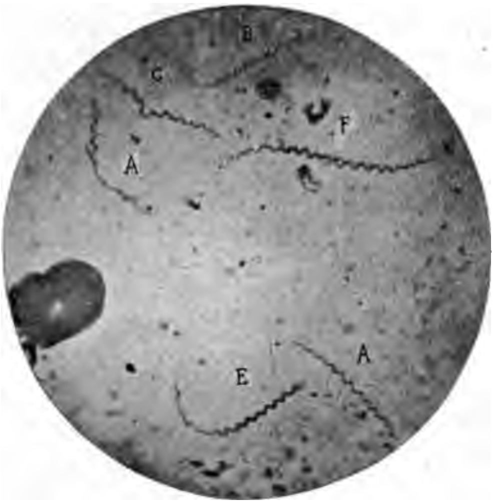


FIG. 171.—Spirochæte pallida.

Staining Methods.—Excellent results are obtained with Goldhorn's stain (which see). To this end the smears, on slides or covers, are covered with the dye for three or four seconds, when the excess is drained off. The specimens are then introduced *slowly* into clean water with the film side down, permitting in this manner an interaction between the film of adhering dye and the water. The slide is held in this slanting position for another four or five seconds and is next shaken in the water so as to wash off the excess of the dye. The pallida appears of a violet color, which may be changed to bluish black by flooding the preparation for fifteen to twenty seconds with Gram's iodine solution, washing and drying as usual. The examination is conducted with a $\frac{1}{12}$ or $\frac{1}{16}$ immersion lens.

The material should in all cases be obtained by curettage, this being carried so far until a small amount of serum and blood appears, and preferably at the edge of the lesion. The serous fluid is then spread upon slides or covers in the usual manner. The organisms are most numerous in moist papules and chancres (when the curettage is carried out at the edge of the lesion). In roseolar scrapings the search is frequently disappointing.

Giemsa's method also furnishes excellent results. I have recommended a dilute alcoholic solution of Victoria blue. Keidel finds that steaming the specimens with this solution for a minute or two furnishes good results.

LITERATURE.—Schaudinn and Hoffmann, Arbeiten aus d. kais. Gesundheitsamte, 1905, vol. xxii, p. 527; Deutsch. med. Woch., 1905, No. 18, p. 711; Berlin. klin. Woch., 1905, May 29, p. 673. Metschnikoff and Roux, Le bulletin méd., May 17, 1905, p. 441. Levaditi, La semaine méd., May 24, 1905, p. 247. Hoffmann, Berlin. klin. Woch., 1905, No. 22, p. 673. Fanoni, Med. News, October, 1905. Babes and Panea, Berlin. klin. Woch., 1905, No. 28, p. 865. Mulzer, ibid., No. 36, p. 1144. Goldhorn, Jour. Exper. Méd., 1906, vol. viii, No. 3.

CHAPTER IX.

THE CEREBROSPINAL FLUID.

According to our present knowledge, the cerebrospinal fluid is secreted by the choroid plexuses into the lateral ventricles. Passing through the foramina of Monro, the third ventricle, and the aqueduct of Sylvius, on the one hand, it reaches the fourth ventricle and enters the cystern-like subarachnoid spaces at the base of the brain, through the foramen of Magendie and the lateral clefts of the fourth ventricle. On the other hand, a certain portion of the fluid reaches the same destination directly through the cleft in the descending horn of each lateral ventricle. The larger portion of the fluid then passes upward through the subarachnoid spaces along the convexity of the brain to the Pacchionian granulations, while the smaller portion enters the vertebral canal through the subarachnoid spaces of the spinal arachnoid membrane.

Within recent years puncture of the vertebral canal has been frequently resorted to, both for therapeutic and diagnostic purposes. The practical value of this method of diagnosis is now beyond question, and it is to be hoped that ere long physicians will resort to spinal puncture in obscure cases of cerebrospinal disease with as little hesitancy as puncture of the thoracic and abdominal cavities is now practised.[1]

The *operative method* to be employed is the following: With the patient placed upon his left side—some observers prefer the sitting posture—and the body bent well forward, a long aspirating needle is introduced upon a level with the lower third of the third or fourth lumbar spinous process, and about 1 cm. to the side of the median line, the needle being directed slightly upward and inward. The depth to which it is necessary to puncture will, of course, vary with the age of the patient. In a child two years of age the vertebral canal may be reached at a depth of 2 cm., while in the adult it is necessary to insert the needle for a distance of from 4 to 8 cm. As soon as the subarachnoid space is reached cerebrospinal fluid will flow from the needle. *Aspiration* should always be avoided.

Some writers have advised that the operation be performed under narcosis; and without doubt this may be necessary at times, particularly when contracture of the dorsal muscles exists. In the majority of cases, however, it is not necessary and local anesthesia will suffice.

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[1] H. Quincke, Verhandl. d. X Cong. f. inn. Med., 1891. A. Hand, " A Critical Summary of the Literature on the Diagnostic and Therapeutic Value of Lumbar Puncture," Amer. Jour. Med. Sci., 1900, vol. cxx, p. 463. A. Stadelmann, "Klinische Erfahrungen mit d. Lumbalpunction," Deutsch. med. Woch., 1897, p. 745.

Amount.—So far as I have been able to ascertain, no observations have been made regarding the amount of fluid which may be obtained by puncture in normal individuals. In all probability, however, this is small. Under pathological conditions the amount may vary from a few drops to 100 c.c., and even more. In general terms it may be stated that the amount is directly proportionate to the degree of intracranial pressure. Exceptions, however, are frequent. Small amounts of cerebrospinal fluid or none at all may thus be obtained when, owing to the formation of a thick exudate or the existence of a cerebral tumor, communication between the basilar subarachnoid spaces of the brain and those of the spinal cord has been interrupted. Whenever, then, symptoms of intracranial pressure exist, while no fluid or minimal amounts only can be obtained by puncture, the conclusion will usually be justifiable that we are dealing with a purulent meningitis or with a tumor of the brain, and more especially of the cerebellum. It should be remembered, however, that the same result may be obtained in cases of obliteration of the aqueduct of Sylvius, or when sclerotic processes involve the foramen of Magendie, which is occasionally observed in certain forms of hydrocephalus. Adhesions of the pia mater to the arachnoid and the dura mater may, by interfering with the flow of cerebrospinal fluid, also lead to the formation of hydrocephalus, but in these cases a tumor can usually be excluded, as the changes in question always develop as sequels to a meningitis. A serous or tuberculous meningitis, as well as acute hydrocephalus and tetanus, can, however, always be excluded when only minimal amounts of fluid are obtained by puncture. The largest amounts, on the other hand, are seen in cases of serous meningitis, tuberculous meningitis, and cerebral tumors, which do not interfere with the circulation of the cerebrospinal fluid. In the epidemic type of meningitis 70 to 80 c.c. can usually be obtained very readily. In epilepsy Pellagrini usually obtained amounts varying between 10 and 15 c.c.[1] Donath gives rather higher figures, up to 60 c.c., and in a tabes case 85 c.c.

Appearance.—Normal cerebrospinal fluid, as well as that obtained in cases of serous meningitis, tuberculous meningitis, hydrocephalus, and tumors of the brain, is perfectly clear, and as a rule colorless unless a small bloodvessel has been punctured, when the fluid may present a slightly reddish tinge. More or less pronounced yellow shades are, however, at times observed. Important from the standpoint of diagnosis is the fact that in cases of hemorrhage into the ventricles pure blood is obtained, while such a result is, of course, a mechanical impossibility in cases of epidural hematoma. In subdural hematoma, on the other hand, blood may also find its way into the subarachnoid space, but the amount is always small, and cannot be compared with that seen in cases of ventricular hemorrhage. Whenever, then, as in traumatic cases with severe cerebral symptoms, the surgeon is confronted with the question whether or not to trephine, puncture of the subarachnoid space may furnish much valuable information. If in such cases no blood at all is found, it may be inferred that an epidural hematoma or a subdural hematoma of slight extent only exists; an operation may then be performed. If, however, pure blood is encountered, it would be justifiable to assume the existence of extensive injury to the brain substance proper, or, in cases in which the history is obscure, an intracerebral hemorrhage with rupture into the ventricles. In such cases the idea of an operation would, of course, be entertained only under exceptional conditions. If, further, the fluid is only tinged with blood, a subdural hematoma probably exists, and an operation should be advised. Accidental hemorrhage, viz., hemorrhage referable to the puncture itself, can be readily recognized, as the first few drops only are then tinged with blood, or the blood appears only after the flow has been definitely established; the amount, moreover, is insignificant.

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[1] La Riforma med., 1901, Ann. 17, vol. ii, p. 638.

Cloudy fluid is obtained in all cases of purulent meningitis unless the disease is limited to a very small area. In the epidemic type, however, it may be quite clear, or but slightly cloudy. Cases of abscess of the brain or sinus thrombosis occur again and again in which the question as to the advisability of operative interference is largely dependent upon the presence or absence of a complicating purulent meningitis. In certain instances a satisfactory conclusion may, of course, be reached without puncture; but in many others this is impossible, and Lichtheim's dictum, that an operation should never be undertaken in such cases unless the integrity of the meninges has been established by spinal puncture, should be borne in mind.

The degree of cloudiness naturally varies in different cases, and while in some instances the character of the fluid is seropurulent, pure, creamy pus may be found in others. Generally speaking, a cloudy fluid indicates the existence of an acute inflammatory process or an exacerbation of a chronic process.

Important, furthermore, is the fact that the fluid in non-inflammatory diseases of the brain, such as tumor or abscess, rarely undergoes coagulation, while this is the rule in all inflammatory diseases. In tuberculous meningitis the coagula are very delicate, and may be well compared with spider-webs extending throughout the fluid, while in purulent meningitis the coagula are somewhat firmer.

Specific Gravity.—The specific gravity of cerebrospinal fluid normally varies between 1.005 and 1.007, corresponding to the presence of from 10 to 15 pro mille of solids. Under pathological conditions variations from 1.003 to 1.012 may be observed, the specific gravity, generally speaking, being higher in the inflammatory than in the non-inflammatory diseases of the brain. From a diagnostic standpoint, however, the determination of the specific gravity is of little value, as numerous exceptions occur to the above rule.

The reaction is always alkaline.

CHEMICAL COMPOSITION OF CEREBROSPINAL FLUID.

An idea of the chemical composition of the cerebrospinal fluid may be formed from the following analyses, taken from Gautier and Zdarek:

	Per cent.
Water	987.00
Albumin	1.10
Fat	0.09
Cholesterin	0.21
Alcoholic and aqueous extract, minus salts Sodium lactate	2.75
Chlorides	6.14
Earthy phosphates	0.10
Sulphates	0.20

ZDAREK'S ANALYSIS.

Water	989.54
Solids	10.45
Organic solids	2.09
Mineral ash	8.35
Albumins	0.76
Ethereal residue	0.35
Aqueous residue	8.22
Sulphuric acid (SO_3)	0.04
Chlorine	4.24
Carbon dioxide	0.49
Potassium oxide	0.16
Sodium oxide	4.29
Mineral ash, insoluble in water	0.16
Glucose	0.10

In addition, urea is at times found, as also a substance which reduces Fehling's solution and gives rise to a brown color when boiled with caustic potash, but which neither undergoes fermentation nor forms an osazone when treated with phenylhydrazin. The substance in question is generally regarded as pyrocatechin. Its amount varies between 0.002 and 0.116 per cent. According to C. Bernard, glucose may also be present, but it is questionable whether this is the case under normal conditions (see below). Nawratzki discovered a reducing substance in his cases, which was demonstrated to be glucose; his subjects, however, were unfortunately not normal, but general paretics with fever. Pyrocatechin was absent. Zdarek[1] reports a recent case of anterior meningocele in an otherwise normal individual in which the fluid reduced Fehling's solution and gave a glucosazone with phenylhydrazin. The substance in question was dextrorotatory, the amount equalling 0.1 per cent. of glucose.

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[1] Zeit. f. phys. Chem., 1902, vol. xxxv, p. 202

Lichtheim claims to have found glucose—by means of the phenylhydrazin test—in all cases of tumor which he examined. In cases of tuberculous meningitis, on the other hand, a positive result was only exceptionally obtained. Quincke also reports that he was able to demonstrate the presence of sugar whenever the liquid obtained was sufficient in amount for the necessary tests. Unfortunately, however, he does not detail his cases. Concetti found no sugar in hydrocephalic fluid.

The experience of other observers does not agree with that of Lichtheim and Quincke; and Fürbringer,[1] who has thus far reported the largest number of spinal punctures, found sugar in only 2 cases of diabetes associated with tuberculosis.

So far as the albuminous bodies are concerned which may be found in the cerebrospinal fluid, serum albumin is said to be present only under exceptional conditions, while normally a mixture of globulin and albumoses is found. The question whether or not mucin may also be present is still undecided.[2]

Under pathological conditions the amount of albumin may vary considerably, and is of diagnostic importance. According to the majority of observers, the figure given in the above analysis is too high, and it is doubtful whether 1 pro mille may be regarded as normal. The lowest values have been obtained in cases of chronic hydrocephalus (traces only), meningitis serosa (0.5 to 0.75 pro mille), and tumors of the brain (traces to 0.8 pro mille); while the largest amounts have been found in chronic hydrocephalus the result of hyperemia (1 to 7 pro mille), and in tuberculous meningitis (1 to 3 pro mille). Nawratzki in recent examinations found amounts varying between 0.047 and 0.170 per cent., but the subjects of his investigation had fever at the time. Mott and Halliburton[3] found three times the normal amount of albumin in paralytics, as also some nucleo-albumin, which does not occur in health. The latter they suppose to come from broken-down Nissl bodies.

Cholin.—According to Gumprecht, the normal cerebrospinal fluid also contains traces of cholin. Donath obtained positive results (using 10 to 20 c.c.) in 15 cases of genuine epilepsy out of 18, three times in 3 cases of Jacksonian epilepsy, once in a case of syphilitic epilepsy, twice in 3 cases of dementia paralytica, once in 2 cases of taboparalysis, ten times in 15 cases of tabes dorsalis, three times in 3 cases of cerebral syphilis, twice in 2 cases of cerebral abscess, once in a case of encephalomalacia, once in a case of spina bifida, once in a case of compression myelitis, once in a case of alcoholic polyneuritis, once in 3 cases of neurasthenia, and once in 3 cases of hystero-epilepsy. Negative results were obtained in 2 cases of hysteria and in multiple cerebrospinal sclerosis. Quantitative estimations were made in 10 cases; the amounts varied between 0.021 and 0.046 per cent.

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[1] Verhandl. d. XV Cong. f. inn. Med., 1901.

[2] Stadelmann, Mitth. a. d. Grenzgebieten d. Med. u. Chir., vol. ii. Comba, Clin. med., 1899 (cited in Arch. d. méd. d. enfants, 1900). Lenhartz, Verhandl. d. XIV Cong. f. inn. Med., 1900.

[3] The Lancet, April, 1901.

Method.—According to Donath,[1] the cerebrospinal fluid (10 to 30 c.c.) is collected in test-tubes, feebly acidified with dilute hydrochloric acid, and evaporated to *dryness* on the water bath. The dark (orange yellow to dark brown) residue is extracted with *absolute* alcohol (99 per cent. is not sufficient), and the filtered solution treated with a solution of platinum chloride in *absolute* alcohol. On standing the chloroplatinate of cholin separates out. This can be identified by its ready solubility in cold water (as contrasted with the very slight solubility of potassium and ammonium platinochloride) and its very characteristic crystals. These are usually serrated and lanceolated or leaf-wreath or rosette shaped, the latter with three or four leaves. Occasionally they are radiate needles, or needles arranged in sheaves (obliquely cut prisms) or hexagonal or rhombic platelets. They are commonly tinged yellow, but if very thin (especially the needles) they appear colorless. The crystals are best obtained by allowing a few drops of their *aqueous* solution to evaporate on a slide.

The alkaline platinochlorides appear as octohedra or tetrahedra, which may have blunt angles; but according to Donath they are never seen with the method as above outlined (using absolute alcohol—alcohol dehydrated with anhydrous copper sulphate and kept over this).

Another delicate reagent for cholin in aqueous solution is phosphotungstic acid. In dilute solutions a white precipitate will form which appears under the microscope as composed of small hexagonal plates or rhomboids. As chloride of potassium and ammonium will also give a precipitate with phosphotungstic acid, the extract in absolute alcohol (see above) should be filtered, the alcohol evaporated, and the residue dissolved in water.

The physiological test for cholin, viz., fall in blood pressure following its intravenous injection in aqueous solution, is usually unnecessary.

Coriat[2] found cholin invariably present in general paresis, also in 1 case of central neuritis, in 2 alcoholic cases with polyneuritis, in 1 of senile dementia, in 1 of senile dementia associated with a tumor in the corpus callosum, in 1 of traumatic organic dementia, also associated with a tumor of the corpus callosum. The largest amounts were found in paresis. Lecithin was found twice by Donath, once in a tabes case and once in Jacksonian epilepsy.

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[1] Med. News, January 21, 1905.

[2] Amer. Jour. Insanity, 1904, No. 4.

MICROSCOPIC EXAMINATION.

Cytology.—Normal cerebrospinal fluid contains either no morphological elements at all or only a small number of lymphocytes (three to eight to a field, with a medium power). Deviations from this normal condition, as has been first shown by Widal, Ravaut, Sicard, and others, may be of marked diagnostic value.

Aside from tuberculous meningitis in which lymphocytosis is practically constant an increased number of lymphocytes has been observed in syphilitic lesions of the central nervous system (general paresis, tabes, cerebrospinal syphilis, syphilitic hemiplegia), in certain cases of herpes zoster, sciatica, and parotitis. Of these the syphilitic cases are most important, but it is to be noted that the increase may be intermittent and paroxysmal. As a rule it is well marked. Lymphocytosis also occurs in lead intoxication and in saturnine encephalopathy it may be quite intense. The same has been noted in African sleeping sickness. Negative results have been obtained in poliomyelitis, syringomyelia, the hemiplegia of old age, polyneuritis, functional neuroses, compression myelitis, cerebral tumors, and epilepsy.

According to Niedner, lymphocytosis is quite constant in syphilitic hemiplegia, while it is inconstant in tabes. Of 9 cases reported by Niedner and Mamlock,[1] lymphocytosis occurred in 5. In general paresis lymphocytosis is very common.

In the epidemic form of cerebrospinal meningitis the predominating cell is the polynuclear neutrophile, excepting in chronic cases where lymphocytes may prevail. This cell also enters into the foreground as recovery occurs.

Donath summarizes his results in 98 cases as follows: In acute and purulent meningitis polynuclear leukocytes prevail; in chronic or less intense processes, especially in tuberculous meningitis, lymphocytes predominate. In the differential diagnosis of syphilitic meningitis, the early stages of tabes and of general paresis, from neurotic conditions and other malignant processes, lymphocytosis points to the first group. In tetanus a large number of polynuclear neutrophiles may also occur.

While in cerebrospinal meningitis referable to the Diplococcus pneumoniæ polynuclear leukocytosis is probably the rule, exceptions occur. Goggia[2] thus reports a fatal case in which daily examinations showed a predominance of the small mononuclear elements throughout the course of the disease.

In connection with cerebral hemorrhage (especially hemorrhage into the ventricles) Sabrazès and Muratet[3] have described the occurrence of large, round, oval, or polyhedral cells, either singly or in plaques, provided each with a single oval nucleus containing several nucleoli. These cells commonly contain red blood corpuscles, often in large numbers, as also crystals and amorphous particles of hematoidin, leukocytic nuclear debris and vacuoles. These cells are macrophages, derived undoubtedly from the endothelial lining of the subarachnoid spaces. Besides, granular structures may be met with which may contain globules of fat, nuclear debris, globules of myelin, red cells, and blood pigment. What these latter cells are is not known. Sabrazès inclines to view them as neuroglia cells.

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[1] Zeit. f. klin. Med., 1904, Heft 1 and 2.

[2] Gaz. d. Osped. e. d. clin., 1905, No. 13.

[3] Soc. d. biol., 1903, pp. 1226 and 1435.

The *technique* employed in the cytological study of the cerebrospinal fluid is the same as in the case of pleural exudates.

Bacteriology.—Very important from a diagnostic standpoint is the fact that pathogenic microörganisms may be found. Lichtheim, Fürbringer, Freyhan, Dennig, Fränkel, and many others since, were thus able to demonstrate the presence of *tubercle bacilli* in a fairly large number of cases of tuberculous meningitis. Some observers, it is true, have been less fortunate, but the fact that Fürbringer found tubercle bacilli in 30 cases out of 37 is certainly significant. Schwarz states that he obtained positive results in 16 out of 22 cases; Slawyk and Manicatide found bacilli in all of 19 cases (sixteen times by direct microscopic examination and three times by the animal experiment) and Koplik found them in 13 out of 14 cases, using centrifugalized material. In order to examine for tubercle bacilli, the fluid should be placed on ice for from six to twenty-four hours, until a slight coagulum has formed, when the fine, spider-web-like threads of fibrin are transferred to a cover-slip, spread in as thin a layer as possible, and stained as described in the chapter on the Sputum. If a centrifugal machine is available, the examination may, of course, be made at once; the chances of finding the bacilli are then also much greater. In every case a large number of specimens should be prepared before the search is abandoned. Only a positive result, however, is of value, and in doubtful cases recourse should be had to the animal experiment.

In the diagnosis of epidemic cerebrospinal meningitis lumbar puncture is of signal value, as the *Diplococcus meningitidis intracellularis* (meningococcus) of Weichselbaum-Jäger can be demonstrated in a large percentage of cases. Councilman thus states that during a recent epidemic of the disease in Boston lumbar puncture was performed in 55 cases, and that in the fluid obtained the diplococci were found on microscopic examination or in culture in 38 cases. The organism was present in all the acute cases, but rarely found in those which ran a more chronic course. The average time from the onset of the disease before spinal puncture was made was seven days in the positive cases and seventeen days in the negative cases. The longest time after the onset in which a positive result was obtained was twentylays. Similar results have also been reached by other observers. k thus found the organism within the first twenty-four hours the onset of the disease and as late as the fifteenth week. In ic cases, however, as Councilman also found, it may escape tion, especially in those of the posterior basic type.

e organism in question is a diplococcus, each half being of about ıme size as the ordinary pathogenic micrococci (Fig. 172). It ıdily stained with the usual dyes, and decolorized by Gram's ɔd. Short chains of from four to six may at times be seen, ıo tetrads and peculiarly swollen forms which are much larger the usual forms. Cultivation is difficult and the organism ly dies out. It grows best upon Löffler's blood-serum mixture, ng round, whitish, shining, viscid-looking colonies, with smooth, ly defined outlines, which may attain a diameter of from 1 to m. in twenty-four hours. Their cultivation upon plain agar,

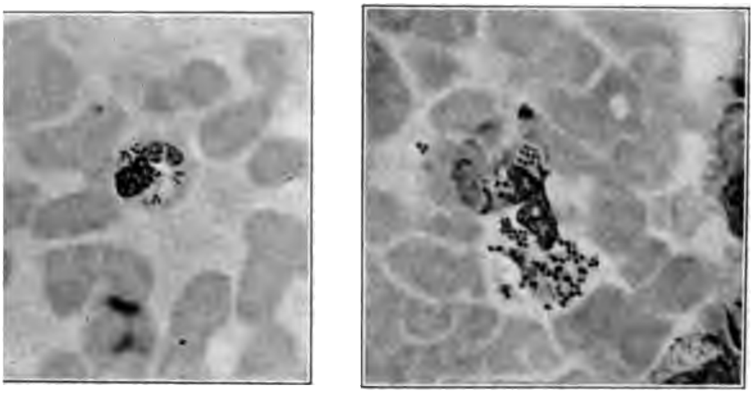

FIG. 172.—Diplococcus meningitidis intracellularis.

rin agar, and in bouillon is less reliable. I have obtained excel- esults by placing a few c.c. of the cerebrospinal fluid in blood- ı tubes and found that the organisms multiplied far more actively ɜ fluid over the medium than in any other way.

order to obtain the best results, it is necessary to use large ınts of the exudate, and to make a number of cultures, as many e organisms are usually dead, or at least will not grow. In ary cover-slip preparations they are often numerous, and are l enclosed in the polynuclear leukocytes. Their number then ɜ considerably. On the one hand, only one or two may be nt in a cell, while in others they may be so closely packed as to ıre the nucleus. During the past winter I examined a specimen ıich the organism was present in groups composed of hundreds, his is rare.

Mixed infections are not uncommon in epidemic cerebrospinal meningitis. Councilman thus found the pneumococcus in 7 cases and Friedländer's bacillus in 1. Terminal infections with staphylococci and streptococci also occur.

In other forms of purulent meningitis a large variety of organisms has been found. Wolf gives the following figures, resulting from an analysis of 174 cases, in which epidemic cerebrospinal meningitis is, however, included: in 44.23 per cent. the pneumococcus was found; in 34.48 per cent. the Diplococcus meningitidis intracellularis; in 3.45 per cent. staphylococci; in 8.03 per cent. streptococci; in 1.13 per cent. the bacillus of Friedländer; in 2.87 per cent. the Bacillus typhosus; in 1.72 per cent. the bacillus of Neumann-Schäffer, and in 2.87 per cent. the Bacillus coli communis, the Bacillus pyogenes fœtidus, the Bacillus aërogenes meningitidis, and the Bacillus mallei; while no bacteria were found in 1.15 per cent. of the cases. In 2 cases Pfeiffer's influenza bacillus has also been encountered in the cerebrospinal fluid during life.

In the African sleeping sickness *trypanosomes* are commonly found in the cerebrospinal fluid, obtained by lumbar puncture. Castellani obtained the organism in 20 cases of 34, and Bruce found it in all of 38 cases (see Blood). The results of these earlier observers have been abundantly confirmed. In many cases, however, the parasites never find their way into the cerebrospinal fluid. They are more frequently found toward the termination of the disease. Large numbers are rare, but if they do occur there is usually an access of temperature. When present, the leukocytes are apt to be increased. There is no relation between the number present in the blood and in the spinal fluid.

Toxicity.—While normal cerebrospinal fluid possesses distinct toxic properties, it has been found that in disease the toxicity may be markedly increased. Bellisari has thus shown that the fluid of individuals suffering from general paresis is more toxic than that of normal individuals, and that this toxicity is at its maximum after an epileptic seizure. Pellegrini further could demonstrate that the cerebrospinal fluid of epileptics is markedly toxic, and that that obtained immediately after a convulsion has a toxic and convulsive power much greater than that obtained at periods far removed from paroxysms. Similar results have been obtained by Dide and Laquepée.

LITERATURE.—W. T. Councilman, "Cerebrospinal Meningitis," Johns Hopkins Hospital Bull., 1898, p. 27; and Phila. Med. Jour., 1898, p. 937. W. T. Councilman, F. B. Mallory, and J. H. Wright, "Epidemic Cerebrospinal Meningitis," Amer. Jour. Med Sci., 1898, p. 252. W. Osler, "The Cavendish Lecture on the Ætiology and Diagnosis of Cerebrospinal Fever," Phila. Med. Jour., 1899, p. 26. E. Stadelmann, "Meningitis Cerebrospinalis," Zeit. f. klin. Med., vol. xxxviii, p. 46. R. Neurath, Centralbl. f. d. Grenzgebiete d. Med. u. Chir., 1897, vol. i. J. Langer, Jahrb. f. Kinderheilk., 1901, vol. iii, p. 91. Pellegrini, Riform. med., 1901, No. 55. Dide and Laquepée, Soc. d. neurol. de Paris, April, 18, 1901.

CHAPTER X.

THE EXAMINATION OF CYSTIC CONTENTS.

CYSTS OF THE OVARIES AND THEIR APPENDAGES.

THE material obtained from cysts of the ovaries or their appendages varies greatly in character. On the one hand, it may be fluid, clear, of low specific gravity, and contain little albumin; while, on the other, it may be dense, viscid, and of colloid appearance. The specific gravity varies between 1.018 and 1.024, owing to the presence of a large amount of albumin.

In addition to smaller amounts of serum albumin and serum globulin the fluid of ovarian cysts contains a considerable quantity of another albuminous substance, which is termed *metalbumin* (Scherer) or *pseudomucin* (Hammarsten). Like Hammarsten's mucoid of transudates, it cannot be directly precipitated with acetic acid, but must be isolated as follows: The fluid in question is freed from coagulable albumins by boiling after acidifying with acetic acid; the filtrate is precipitated with alcohol, the precipitate dissolved in water, dialyzed, and then treated with acetic acid, when the pseudomucin separates out. The substance contains about 30 per cent. of glucosamin.

Paramucin is another albuminous substance which is found in colloid cysts and belongs to the mucinoid bodies. Like the true mucins and the body which occurs in exudates the paramucin is also precipitated by dilute acetic acid. According to Mitjukoff, it contains at least 12.5 per cent. of a reducing substance.[1]

Test for Pseudomucin.—The fluid is mixed with three times its volume of alcohol and set aside for twenty-four hours, when it is filtered and the precipitate suspended in water. This is again filtered and the filtrate tested in the following manner: (1) A few cubic centimeters are boiled, when in the presence of metalbumin the liquid will become cloudy, without the formation of a precipitate. (2) With acetic acid no precipitate is obtained. (3) Upon the application of the acetic acid and potassium ferrocyanide test the liquid becomes thick and assumes a yellowish color. (4) When boiled with Millon's reagent a few cubic centimeters of the filtrate will yield a bluish-red color, while the addition of concentrated sulphuric acid, without boiling, gives rise to a violet color.

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[1] Literature dealing with pseudomucin and paramucin: *Pseudomucin:* Hammarsten, Zeit. f. phys. Chem., 1882, vol. vi, p. 194. Pfannenstiel, Arch. f. Gynäk., 1890. Zängerle, Münch. med. Woch., 1900. *Paramucin:* Mitjukoff, Arch. f. Gynäk., 1895. Panzer, Zeit. f. phys. Chem., 1899, vol. xxviii. Leathes, Arch. f. exper. Path. u. Pharmak., 1899, vol. xliii.

The color of cystic fluids may vary from a light straw to a reddish brown, or even a chocolate; the latter color may be observed when hemorrhage has taken place into the cyst.

Of morphological elements, ovarian cysts contain red blood corpuscles, leukocytes, and at times fatty granules in large numbers, crystals of cholesterin, hematoidin, and fatty acids. Most important, however, from a diagnostic standpoint is the presence of cylindrical or prismatic, ciliated epithelial cells, derived from the internal lining of the cyst, in the presence of which the diagnosis may be definitely made (Fig. 173). At times such cells cannot be demonstrated, as they may have undergone fatty degeneration; moreover, if the epithelium lining the cyst is squamous in character, it may be difficult, if not impossible, to arrive at a satisfactory conclusion from an examination of the morphological elements alone. *Colloid concretions*, which may vary in size from several micromillimeters to 0.1 mm., are occasionally observed, and more particularly in colloid cysts. They may be recognized by their irregular form, homogeneous appearance, slightly yellow color, and delicate outlines.

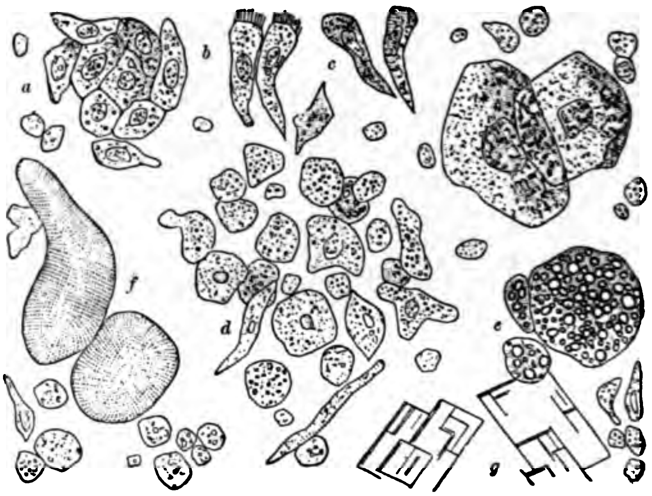

FIG. 173.—Contents of an ovarian cyst: *a*, squamous epithelial cells; *b*, ciliated epithelial cells: *c*, columnar epithelial cells; *d*, various forms of epithelial cells; *e*, fatty squamous epithelial cells; *f*, colloid bodies; *g*, cholesterin crystals. (Eye-piece III, obj. 8 A, Reichert.) (v. Jaksch.)

In dermoid cysts, epidermal cells and occasionally hairs are observed.

The differential diagnosis of ovarian, parovarian, and fibrocystic (uterine) cysts cannot always be made from the character of the fluid withdrawn by puncture, but at times it is possible. The most important points of difference are here given: (1) The fluid in ovarian cystomas is usually more or less viscid, and often contains non-nucleated granular corpuscles of about the size of leukocytes, the granules of which do not dissolve in acetic acid nor disappear when treated with ether. In all probability they are free nuclei; in the United States they are often called Drysdale's corpuscles. (2) In parovarian cysts the fluid is thin, watery, of low specific gravity (under 1.010), and contains very few morphological elements. Cylindrical epithelium is very rarely found during life in the fluid withdrawn by aspiration from either ovarian or parovarian cysts. (3) The fluid from fibrocystic tumors of the uterus is thin, watery, and coagulates spontaneously, while that from ovarian and parovarian cysts never coagulates spontaneously unless blood is present. Fibrocystic tumors of the uterus have no epithelial lining.

Of special interest are those cases of ovarian cysts in which in the course of typhoid fever infection of the cystic contents occurs with the corresponding organism.[1]

HYDATID CYSTS.

The normal fluid in hydatid cysts is clear like water, neutral (sometimes faintly acid or alkaline), of a specific gravity of 1.000 to 1.015, and rich in sodium chloride. By transmitted light it is faintly opalescent. It contains no albumin or only a trace of it. Succinic acid or sugar may be present in small amount. Sodium chloride may be recognized by evaporating a drop of the liquid on a slide, when the characteristic crystals of the salt will be found. Succinic acid may be demonstrated by acidifying a small amount of the fluid with hydrochloric acid, and evaporating to dryness. The residue is extracted with ether and the ether evaporated; the aqueous solution of the second residue, in the presence of succinic acid, will yield a rust-colored, gelatinous precipitate when treated with a few drops of a solution of ferric chloride. A sediment, if present, is composed chiefly of scolices, debris of parenchyma, calcareous particles, and hooklets. Hematoidin crystals may be found if blood has entered the cyst. Where tapping or exploratory puncture has been employed, albumin may afterward be found in greater quantity, as also in degenerating and suppurating cases. With the death of the hydatid, changes of a degenerative nature take place, the fluid altering greatly in character. It becomes more turbid, fatty globules may be found with granular cells, and typical crystals of cholesterin. The contents may become of putty-like consistence and greasy, containing the remains of the gelatinous membranes which may be floated out in water. Should calcification ultimately occur, hooklets may be found on rubbing up the material with water in a mortor.

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[1] M. J. Lewis and R. G. Le Conte, Amer. Jour. Med. Sci., 1902, vol. cxxiv, p. 590.

When suppuration takes place, polymorphonuclear leukocytes are first found between the cyst and its adventitious capsule; the cysts ultimately may become softened and burst, membranes, scolices, and hooklets floating about in the pus. (See also Sputum.)

HYDRONEPHROSIS.

The diagnosis of hydronephrosis can usually be made without difficulty if a sufficient amount of fluid can be obtained; the presence of urea and uric acid in *notable quantities*, as well as of renal epithelial cells, which latter especially should be sought for, is quite characteristic. *Small* amounts of uric acid, however, may also be present in ovarian cysts.

PANCREATIC CYSTS.

These cysts may be recognized by the fact that the fluid possesses the power of digesting albumin in alkaline solution. A small amount of the liquid is added to a few c.c. of milk, when after precipitation of the casein the biuret test is applied; a positive reaction indicates the presence of *trypsin*. Unfortunately, however, the test does not always yield positive results, even if the fluid in question is derived from a pancreatic cyst, as the trypsin is apparently destroyed in the course of time. The larger the cyst, the less likely will it be possible to obtain the reaction. A positive result is hence only of value, while a negative result does not exclude the existence of the disease.[1]

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[1] Karewski, Deutsch. med. Woch., 1890, vol. xvi, pp. 1035 and 1069. Hofmeister, Prag. med. Woch., 1891, vol. xvi, pp. 365 and 377 (see Gussenbauer). v. Jaksch, Zeit. f. Heilk., 1888, vol. ix, p. 126 (see Wölfler).

CHAPTER XI.

THE SEMEN.

THE ejaculated semen is a mixture of the secretions furnished by the testicles, the prostate gland, the seminal vesicles, and the glands of Cowper.

GENERAL CHARACTERISTICS.

Semen is white or slightly yellowish in color, semifluid, sticky, and of an opaque, non-homogeneous, milky appearance, which is due to the presence of white, opaque islets floating in the otherwise clear fluid; these consist almost entirely of the specific morphological elements of the semen, the spermatozoa. Its odor, which strongly resembles that of fresh glue, is characteristic, and is owing to the presence of *spermin*. It is generally attributed to an admixture of prostatic fluid, as the semen obtained from the vasa deferentia is odorless. According to Robin, however, this odor is produced only at the moment of ejaculation, and cannot be ascribed to any single one of the secretions present. The reaction of human semen is slightly alkaline, and its specific gravity greater than that of water, in which it sinks to the bottom.

CHEMISTRY OF THE SEMEN.

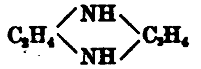
Accurate analyses of human semen or of mammalian semen do not exist, and only the old analyses of Vauquelin and Kölliker can be given:

	Man.	Horse.	Ox.
Water	90	81.90	82.10
Albuminous material	6	....	15.30
Extractives		16.45	
Ethereal extract		....	2.20
Mineral material	4	1.61	2.60

The mineral matter consists largely of calcium phosphate.

If semen is kept, or if it is slowly evaporated, crystals of phosphate of spermin separate out, which are commonly known as Böttcher's crystals, and which were long regarded as identical with the so-called Charcot-Leyden crystals that are found in the sputum of bronchial asthma, in the blood of leukemia, in the stools in cases of helminthiasis, etc.

Spermin is a basic substance, and, according to Ladenburg and Abel, is closely related to, if not identical with, diethylene diamin (piperazin):



$$C_2H_4\begin{matrix}\diagup NH \diagdown \\ \diagdown NH \diagup\end{matrix}C_2H_4$$

The phosphate crystallizes in the form of monoclinic four-sided spindles or prisms, which appear as flattened needles of variable size. Some are scarcely visible even with a fairly high power of the microscope, while others attain the length of 40 μ to 60 μ. The substance is soluble in formalin, thus differing from the Charcot-Leyden crystals. In water it dissolves with difficulty; it is slowly soluble in acids and alkalies, even in ammonia, while it is insoluble in alcohol, ether, chloroform, and dilute saline solution. Florence's reagent (see below) colors the crystals a bluish black. According to Cohn, the Böttcher crystals are formed exclusively in the prostate gland, the gland itself furnishing the basic component, while the necessary phosphoric acid is derived from other portions of the reproductive apparatus.[1]

MICROSCOPIC EXAMINATION OF THE SEMEN.

Upon microscopic examination normal semen is seen to contain innumerable, actively moving, thread-like bodies, measuring from 50 μ to 60 μ in length—the *spermatozoä.* These consist of an egg-shaped head, when seen from above, which is from 3 μ to 5 μ in length, the broader end being dircted anteriorly; a middle portion, 4 μ to 6 μ in length, with which the head is united by its smaller end; and a posterior piece or tail, into which the middle piece gradually fades (Fig. 170).

In addition to the spermatozoa a few hyaline bodies are seen which are derived from the seminal vesicles; further, numerous small, pale granules of an albuminous nature (lecithalbumin), some testicular and urethral epithelial cells, lecithin corpuscles, and so-called prostatic or *amyloid corpuscles,* which at first sight resemble starch granules in appearance, owing to their concentric striations. A few leukocytes and occasionally a few red corpuscles may also be found.

PATHOLOGY OF THE SEMEN.

The study of the semen has received little attention from clinicians, and gynecologists frequently hold the wife responsible for sterility when an examination of the husband's semen would—according to Kehrer,[1] in 40 per cent.—reveal an absence of spermatozoa, constituting the condition usually spoken of as *azoöspermatism*. This may be temporarily observed following venereal excesses, when the fluid finally ejaculated is almost entirely of prostatic origin; their absence then possesses no significance, but persistent azoöspermatism must of necessity be associated with sterility.[2]

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[1] Th. Cohn, "Zur Kenntniss d. Spermas," Centralbl. f. allg. Path. u. path. Anat., vol. x, pp. 940 and 949.

Cases have been recorded in which, notwithstanding the presence of spermatozoa and apparently normal sexual conditions in both husband and wife, sterility existed nevertheless, but in which it was observed that the spermatozoa lost their motile power almost immediately after ejaculation. Under normal conditions, following intercourse actively moving spermatozoa may be found in the vagina after hours, days, and even weeks.

Whenever it is deemed advisable to make an examination of the semen, this should be done immediately following ejaculation, or as soon as possible thereafter. The material should be placed in a test tube and this immersed in lukewarm water until it can be examined. Note should then be taken, not only of the presence, but also of the degree of motility of the spermatozoa, a drop of the semen being examined directly with the microscope.

Bloody semen, constituting the condition spoken of as *hemospermia*, has been observed on several occasions. It may follow excessive sexual indulgence, but may also occur in connection with gonorrheal epididymitis. The blood is readily recognized upon microscopic examination.[3]

THE RECOGNITION OF SEMEN IN STAINS.

In medicolegal cases the physician may be called upon to decide whether or not certain stains on body-linen are caused by spermatic fluid, whether or not a rape has been committed, etc. In such cases it is frequently only necessary to examine a drop of the vaginal fluid in order to arrive at a positive result at once. At other times recourse must be had to the following method: A fragment of the linen or scrapings from the vulva or vagina are placed in a watch-crystal and allowed to soak for at least one hour in from 27 to 30 per cent. alcohol, when a bit of the material is teased in a solution of eosin in glycerin (1 to 200), and examined. The heads of the spermatozoa are thus stained a deep red, while the tails, which are often broken, exhibit a pale-rose tint, and can readily be distinguished from vegetable fibers, which do not take the stain at all. A positive statement can thus be made in every case, even after months and years, as spermatozoa not only resist the action of reagents, but also the process of putrefaction; this is probably owing to the large proportion of mineral matter which enters into their composition, and which ensures the preservation of their form. Instances have been recorded in which it was possible to demonstrate spermatozoa in stains after eighteen years.

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[1] Beiträge z. klin. u. exper. Gynäk., 1879, vol. ii, Giessen.

[2] Fürbringer, Zeit. f. klin. Med., 1881, vol. iii, p. 310.

[3] Feleki, Centralbl. f. Krankh. d. Harn- u. Sexualorgane, 1901, vol. xii, p. 506.

The semen test of Florence[1] has attracted much attention, and may be recommended in doubtful cases; only a negative result, however, is of value (see below). It is based upon the observation that very characteristic crystals of *iodospermin* are formed when spermatic fluid is treated with a solution of iodopotassic iodide containing 1.65 grams of pure iodine and 2.54 grams of potassium iodide, dissolved in 26 c.c. of water. When a drop of this solution is added to a drop of spermatic fluid or an aqueous extract of a seminal stain, dark-brown crystals of iodospermin separate out at once, and may be readily recognized under the microscope. They occur in the form of long rhombic platelets or fine needles, often grouped in rosettes, but also occurring singly or as twin crystals. The examination with the microscope should be made at once after the addition of the reagent, as the crystals disappear on standing.

As the reaction may also be obtained in cases of azoöspermatism, and with pure prostatic secretion, while a negative result is obtained with the fluid from spermatoceles, it is manifest that the test is not applicable for the determination of the presence or absence of spermatozoa *per se*. Posner[2] states that he obtained similar crystals when the test was applied to a glycerin extract of ovaries.

More recently Richter[3] has shown that Florence's reaction is also obtained with a decomposition product of lecithin, viz., cholin, which would explain the observation that better results are commonly obtained with dried semen than with fresh material. But it follows also that the reaction cannot be a specific semen reaction, and Richter accordingly concludes that a negative result only is of value, and indicates that the material under examination is not semen. He states that he obtained positive results with vaginal and uterine mucus, with decomposing brain substance, and other organs as well. In confirmation of Richter's results, Bocarius[4] has demonstrated that the so-called iodospermin is in reality an iodized product of cholin and not of spermin.

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[1] Du sperme et des taches de sperme en médecine legale, Arch. d'Anthrop. crimin., vols. x and xi.

[2] "Die Florence'sche Reaktion," Berlin. klin. Woch., 1897, p. 602.

[3] "D. mikrochemische Nachweis v. Sperma," Wien. klin. Woch., 1897, p. 569.

[4] Zeit. f. phys. Chem., 1902, vol. xxxiv, p. 339.

CHAPTER XII.

VAGINAL DISCHARGES.

GENERAL CHARACTERISTICS.

The secretion which is normally furnished by the vaginal glands is small in amount, and just sufficient to keep the mucous membrane moist. It is a clear or somewhat milky-looking, semiliquid material, in which numerous epithelial laminæ may be found. It has been stated that the reaction of the vaginal secretion in virgins is *invariably* acid, while an alkaline reaction is the rule in the *déflorées*. During pregnancy, however, the secretion is probably always acid. In 500 cases which Krönig examined in this direction an alkaline reaction was never observed. According to Zweifel,[1] the vaginal secretion contains traces of trimethylamin, to which its peculiar odor is probably due.

Microscopically, numerous epithelial cells, mucous corpuscles, a few large mononuclear leukocytes, cellular detritus, and bacteria are found. Döderlein[2] has described a non-pathogenic bacillus or a group of bacilli which are characterized by the fact that they give rise to marked acid fermentation of sugar, and he regards these organisms as the only ones which are constantly present in the normal vagina. Krönig and Menge, however, state that they are often absent. These observers have found, on the other hand, that under normal conditions there are various bacilli and cocci present which belong to the class of obligatory anaërobes, and are likewise non-pathogenic. Unfortunately they have not described these organisms in detail. Near the outlet they found bacteria which may be cultivated upon alkaline aëorbic culture media, but which are usually absent in the upper portion of the vagina.

It is important to note that various diplococci may also be found under normal conditions, and care should be taken not to confound these with gonococci. Like the gonococci, they are decolorized by Gram's method. If the characteristics of the former be borne in mind, however, mistakes may probably always be avoided; in married women and in children it is best to make the diagnosis of gonorrhea only when the gonococcus has been isolated by cultivation.

The question whether or not pathogenic bacteria *may* occur in the normal vagina of pregnant or non-pregnant women, may be answered in the affirmative; but with the exception of the gonococcus they are not often seen.[1] Bergholm[2] thus examined the vaginal secretion of 40 pregnant women, and was unable to obtain organisms pathogenic for animals in a single case. There were no pyogenic staphylococci, no streptococci, and no colon bacilli.

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[1] Arch. f. Gynäk., 1881, vol. xviii, p. 359.
[2] Ibid., 1887, vol. xxxi, p. 412.

The vaginal secretion has been shown to possess powerful bactericidal properties, so that pathogenic organisms, even when artificially introduced into the vagina, are rapidly killed. Krönig thus found that the Bacillus pyocyaneus disappears from the vagina of pregnant women in from ten to thirty hours, the staphylococci in from six to thirty-six hours, and the Streptococcus pyogenes within six hours. Important from a practical standpoint is the fact that the bacteria disappeared less rapidly when irrigation of the vagina with water or even antiseptics was employed.

Of animal parasites, the *Trichomonas vaginalis* is occasionally encountered in the vaginal discharge. The organism is identical with the trichomonas found in the feces and in the urine. In the United States it is not so common as among the peasant population of Central Europe. As far as is known, the organism is of no pathological significance. From a medicolegal standpoint, however, its presence may not be unimportant, as cases are on record in which trichomonades have been confounded with spermatozoa. In my judgment, however, such a mistake can only occur if the observer is totally without training in microscopy.

The possible presence of the Anguillula aceti in the vaginal discharge has been pointed out by Billings, Miller, and Stiles. Stiles has suggested that it may be introduced into the vagina by injections of vinegar-water taken with the object of preventing conception.

VAGINAL BLENNORRHEA.

In physiological conditions an increased vaginal secretion is observed during sexual excitement, just preceding and at the beginning of menstruation, and during pregnancy, when a profuse blennorrhea is frequently seen, which sometimes assumes a virulent character. The secretion under such conditions readily becomes purulent. When not dependent upon a gonorrheal infection the secretion is thicker than normal, white, and creamy. At times also the vaginal catarrh observed in pregnancy is complicated with mycosis, when white or yellowish-gray patches may be seen at the orifice of the vagina; the latter may, indeed, be filled with particles which consist entirely of fungi.

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[1] Döderlein, Das Scheidensecret, Leipzig, 1892. J. W. Williams, Amer. Jour. Obstet., 1898, vol. xxxviii; Trans. Amer. Gyn. Soc., 1898; Amer. Jour. Obstet., 1898.

[2] Arch. f. Gynäk., 1902, vol. lxvi, Heft 3.

MENSTRUATION.

At the beginning of menstruation, as has been pointed out above, an increase in the amount of vaginal secretion is observed, in which leukocytes, prismatic epithelial cells coming from the uterus, as well as the usual vaginal cells, may be seen upon microscopic examination. Later the secretion becomes sanguineous in character, and finally only epithelial cells, leukocytes, and granular detritus are encountered, the cells usually showing evidence of fatty degeneration. The amount of blood lost at each menstrual period amounts to about 200 grams in perfectly healthy females.

THE LOCHIA.

The lochia during the first day following parturition are red in color—the *lochia rubra*—and emit the characteristic sanguineous odor. At this time a microscopic examination will reveal an abundance of red corpuscles, some leukocytes, and a variable number of epithelial cells, which are almost exclusively of vaginal origin. On the second and third days the number of red corpuscles diminishes, while the leukocytes increase in number. Still later the diminution in the red and the increase in the white corpuscles become more marked, and the discharge at the same time assumes a grayish or white color, until about the tenth day the red corpuscles have almost entirely disappeared, while the leukocytes and epithelial cells are abundant. Finally, the secretion becomes thicker, mucoid, and milky white in color—the *lochia alba*—which condition may persist for from three to four weeks in nursing women, and still longer in those who do not nurse, until finally the normal secretion is again established. Numerous bacteria are encountered in the lochia, and it is curious to note that among these pus organisms are quite constantly present without giving rise to symptoms. When a portion of the placenta or membranes have been retained the lochia soon give off a fetid odor, and assume a dirty brownish color; the retention of blood clots alone may also produce this result. In such cases the lochia swarm with bacteria of all kinds.[1]

VULVITIS AND VAGINITIS.

In cases of vulvitis and vaginitis a marked increase is observed in the number of the leukocytes and epithelial cells, the character of the latter depending essentially, of course, upon the portion of the genital tract affected. Red corpuscles are also met with at times; their number generally stands in a direct relation to the intensity of the inflammatory process. In some instances epithelial casts of the entire vagina have been observed, constituting the condition termed *vaginitis exfoliativa*. The condition, however, is rare.

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[1] Döderlein, loc. cit. Thomen, Centralbl. f. d. med. Wiss., 1890, vol. xxviii, p. 537; and Arch. f. Gyn., 1889, vol. xxxvi, p. 231.

In mycotic vaginitis leptothrices have been found by v. Herff.[1]

The discharge of large amounts of pure pus through the vagina points to perforation of an abscess of the genital organs or of the neighboring structures into the uterus or the vagina; it is of rare occurrence. Much more common is the discharge of fecal matter or of urine through this channel, indicating the existence of a vaginorectal or vaginovesical fistula.

MEMBRANOUS DYSMENORRHEA.

While ordinarily, during menstruation, shreds of desquamated uterine lining are frequently encountered, it is rare to meet with large pieces or complete casts of the uterus, the elimination of which is usually associated with the symptoms of a severe dysmenorrhea, constituting the condition spoken of as *membranous dysmenorrhea*.

CANCER.

While the diagnosis of malignant growth of the uterus is probably never based upon a microscopic examination of the vaginal discharge alone, it may be mentioned that in advanced cases this is possible, as fragments of an epithelioma of the cervix, for example, may frequently be detected upon microscopic examination. In suspected cases small pieces of tissue should be excised and examined according to usual histological methods.[2]

GONORRHEA.

In suspected cases of gonorrhea an examination of the vaginal and urethral discharge for the presence of gonococci is important, as it is practically impossible to diagnosticate this condition positively in any other manner. Care should be taken, however, not to confound the diplococci which may be normally present in the urethra and vagina with gonococci. (See chapter on the Urine.) Unfortunately, however, excepting in fairly acute cases, these examinations are rather unsatisfactory. There can be no doubt that in many cases, which unquestionably are gonorrheal, the ordinary microscopic examination is negative. Better results may possibly be reached if the examination is made twenty-four hours following the injection of gonococcus vaccine (dose, 10,000,000 organisms).

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[1] Centralbl. f. Bakter., 1895, p. 750.

[2] T. S. Cullen, Cancer of the Uterus, Appleton & Co., 1900.

ABORTION.

In cases of abortion it is often possible to discover *chorion villi* in the expelled blood-clots which present the characteristic capillary network (Fig. 174), and often manifest signs of advanced fatty degeneration. Important also from a diagnostic point of view is the presence of *decidual cells* (Fig. 175), which are characterized by their large size, their round, polygonal, or spindle-like form, and their characteristic nuclei and nucleoli.

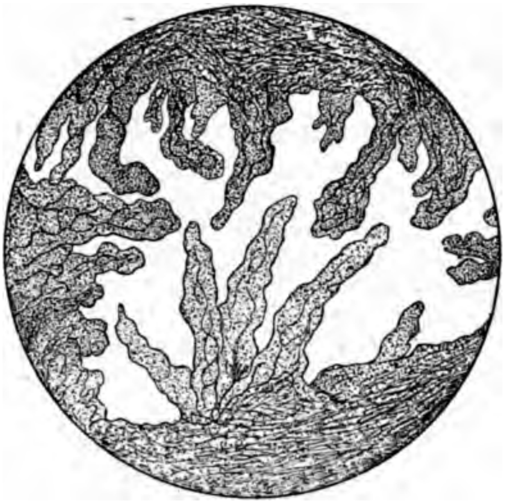

FIG. 174.—Chorion villi.

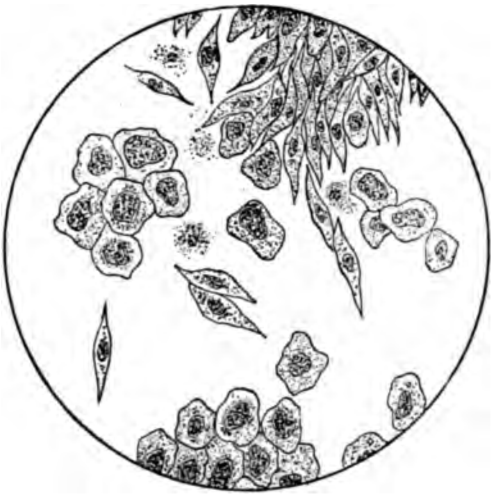

FIG. 175.—Decidual cells.

CHAPTER XIII.

THE SECRETION OF THE MAMMARY GLANDS.

THE SECRETION OF MILK IN THE NEWBORN.

A SECRETION from the mammary glands of the male is observed only in the newborn, if we except those rare cases in which adult males were known to suckle infants. The fluid in question, which may also be obtained from the female infant, is termed "Hexenmilch" (witches' milk) by the Germans. Qualitatively it has the same composition as milk, but may manifest considerable quantitative variations.

COLOSTRUM.

Aside from those curious instances in which a secretion of milk has been observed in non-pregnant women, mammary activity is essentially connected with the physiological phenomena of pregnancy and parturition. Often as early as the third month a small drop of a serous-looking fluid can be obtained from the nipple by pressure upon the breasts. Immediately after delivery a variable amount of fluid is secreted, which is watery, semi-opaque, mucilaginous, and of a yellowish color. To this secretion, as well as to that observed during pregnancy, the term colostrum has been applied. It is distinguished from true milk by its physical characteristics and by the presence of a greater proportion of sugar and salts. The fluid, moreover, coagulates upon boiling. An idea may be formed of its composition from the appended table:

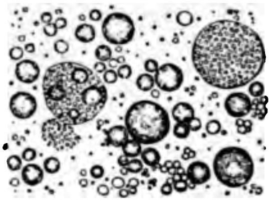

FIG. 176.—Colostrum of a woman in sixth month of pregnancy. (Eye-piece III, obj. 8 A, Reichert.) (v. Jaksch.)

	4 weeks before birth.		17 days before birth.	9 days before birth.	24 hours after birth.	2 days after birth.
	I.	II.				
Water . .	945.2	852.0	851.7	858.8	843.0	867.9
Solids . . .	54.8	148.0	148.3	141.2	157.0	132.1
Casein . .	.....	.....	.....	.....	.....	21.8
Albumin . .	28.8	69.0	74.8	80.7		
Fat . . .	7.3	41.3	30.2	23.5	.....	48.6
Lactose . .	17.3	39.5	43.7	36.4	.....	61.0
Salts . . .	4.4	4.4	4.5	5.4	5.1	

Upon microscopic examination fat droplets, a few leukocytes, some epithelial cells, and so-called *colostrum corpuscles* are found. The latter are highly refractive bodies, of irregular size, whose interior is filled with fatty granules (Fig. 176).

LITERATURE.—G. Woodward, Jour. Exper. Med., vol. ii, p. 217.

THE SECRETION OF MILK PROPER, IN THE ADULT FEMALE.

The secretion of milk proper usually begins about the third day following parturition, and may continue for a variable length of time. On the one hand, the amount of milk secreted may be so small as to be insufficient for the needs of the child, so that lactation may have to cease after several days; on the other hand, women are not infrequently seen who nurse their children for two years and even longer and who may furnish four liters a day. Usually infants are nursed until six or seven teeth have appeared, which period varies with the individual child, averaging about the eleventh month.

HUMAN MILK.

Human milk is of a bluish color, and differs in this respect from the milk of cows. Its reaction is alkaline. The specific gravity may vary between 1.026 and 1.035, one between 1.028 and 1.034 being the most common. The amount of milk secreted in twenty-four hours varies from 500 to 1500 c.c. Microscopically, it is a fairly homogeneous emulsion of fat, and is practically destitute of cellular elements. From the following table an idea may be formed of its chemical composition:

	Biehl.	Gerber.	Christenn.	Pfeiffer.	Pfeiffer.	Mendes de Leon.
Water	876.00	891.00	872.40	892.00	890.60	877.90
Solids	124.00	109.00	127.60	108.00	109.40	
Albumin	22.10	17.90	19.00	16.13	17.24	25.30
Fat	38.10	33.00	43.20	32.28	29.15	38.90
Lactose	60.90	53.90	59.80	57.94	59.92	55.40
Salts	2.90	4.20	2.60	1.65	2.09	2.50

Upon comparing this table with the following analysis of cows' milk it will be seen that the latter contains more albumin and less sugar than human milk. Human milk, moreover, is relatively deficient in mineral matter, and especially in calcium salts and phosphoric acid:

Water	874.2	
Solids	125.8	
Casein	28.8	34.5
Albumin	5.3	
Fat	36.6	
Lactose	48.1	
Salts	7.1	

Of inorganic salts human milk contains about 0.7 pro mille of potassium (K_2O), 0.2 of sodium, 0.3 of calcium, 0.06 of magnesium, from 3.52 to 7.21 mgrms. of iron, about 0.4 pro mille of phosphoric acid, and 0.4 of chlorine.,

The albumins found in milk plasma are casein, lactoglobulin, and lactalbumin. It is claimed by some observers that the casein of human milk differs from that obtained from cows' milk. The casein coagula in human milk are not so large and dense as those observed in cows' milk. Human casein, moreover, is not so readily precipitated by acids and salts; it does not always coagulate upon the addition of rennet ferment, and while it may be precipitated by the gastric juice, it is readily dissolved by an excess.

The question whether or not normal human milk contains microorganisms may now be answered in the affirmative. There can be no doubt, however, that the milk as it is secreted by the healthy gland is sterile, but upon passing along the lacteal ducts in the nipple it is always contaminated by the Staphylococcus epidermidis albus (Welch). This microörganism must be regarded as a constant inhabitant of the skin, and is the only one of the cutaneous bacteria which penetrates the deeper layers of the epidermis and the glandular appendages of the skin. It is thus apparent why this organism is so constantly met with, and is practically the only one found in normal human milk. Exceptionally the Staphylococcus pyogenes aureus is found.

THE MILK IN DISEASE.

The chemistry of the milk in pathological conditions has received little attention. It appears, however, that the milk of women when ill usually contains less fat, and that the proportion of lactose is diminished. In cases of jaundice the presence of bile pigment and of biliary acids has not been satisfactorily demonstrated. According to Friedjung,[1] a subnormal amount of iron is usually found in the milk when nurslings do not thrive on apparently normal milk. In cases of mammary tumors bloody secretion has been observed in rare cases, the nipple itself being intact.

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[1] Arch. f. Kinderheilk., vol. xxxi, Heft 1 u. 2.

Microscopically, an admixture of leukocytes is observed in various diseases of the breasts, and especially in cases of abscess. Of pathogenic microörganisms, streptococci may be found in cases of puerperal fever; more commonly, however, they are absent. The typhoid bacillus has occasionally been seen in cases of typhoid fever, and it is interesting to note that the specific agglutinins of typhoid fever have been found in the milk. Pneumococci have been obtained from the milk of pregnant women affected with lobar pneumonia. The important question whether or not tubercle bacilli are eliminated in the milk in cases of phthisis cannot be definitely answered. In cows such an occurrence is certainly common, even when there is no demonstrable tuberculous lesion of the udder. So far as I have been able to ascertain, however, tubercle bacilli have never been found in human milk.[1]

A blue and a red color have been observed in the milk of cows, owing to the presence of the Bacillus pyocyaneus and the Micrococcus prodigiosus, respectively.

A chemical examination of human milk should always be made when it is apparent that the nutrition of the baby is below normal. Valuable dietetic suggestions may thus be obtained. In other cases, as when the mother is unwilling or unable to nurse her child beyond a certain period, a knowledge of the composition of her milk will enable the physician to give specific instructions regarding the proper modification of cows' milk. If a wet-nurse is to be employed, her milk should likewise be examined. Most important is the determination of the specific gravity and of the amount of fat. The former may vary between 1.029 and 1.033. The amount of fat should not be less than 3 per cent.

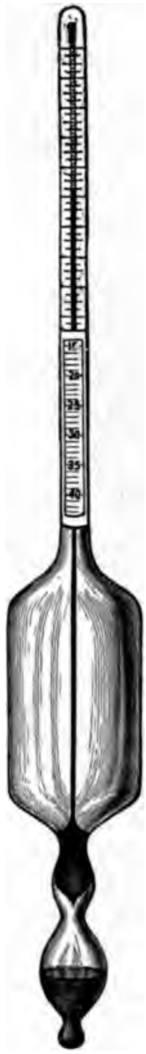

Fig. 177.—Quevenne's lactodensimeter.

Determination of the Specific Gravity.—The specific gravity is best determined with the lactodensimeter of Quevenne (Fig. 177). As the instrument is graduated for a temperature of 60° F., it is necessary to correct the specific gravity when the temperature is above or below this point. In the following tables the corrected specific gravity may be found corresponding to temperatures ranging from 46° to 75° F.:

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[1] Escherich, Fortschr. d. Med., 1885, vol. iii, p. 321. Karlinski, Wien. med. Woch., 1888, vol. xxxviii, No. 28. Ott, Prag. med. Woch., 1892, vol. xvii, p. 145. Cohn u. Neumann, Virchow's Archiv, 1880, vol. cxxvi, p. 187.

Corrections for Temperature.

Specific gravity.	Degrees of thermometer (Fahrenheit).									
	46	47	48	49	50	51	52	53	54	55
1020	19.0	19.1	19.1	19.2	19.2	19.3	19.4	19.4	19.5	19.6
1021	20.0	20.0	20.1	20.2	20.2	20.3	20.3	20.4	20.5	20.6
1022	21.0	21.0	21.1	21.2	21.2	21.3	21.3	21.4	21.5	21.6
1023	22.0	22.0	22.1	22.2	22.2	22.3	22.3	22.4	22.5	22.6
1024	22.9	23.0	23.1	23.2	23.2	23.3	23.3	23.4	23.5	23.6
1025	23.9	24.0	24.0	24.1	24.1	24.2	24.3	24.4	24.5	24.6
1026	24.9	24.9	25.0	25.1	25.1	25.2	25.2	25.3	25.4	25.5
1027	25.9	25.9	26.0	26.1	26.1	26.2	26.2	26.3	26.4	26.5
1028	26.8	26.8	26.9	27.0	27.0	27.1	27.2	27.3	27.4	27.5
1029	27.8	27.8	27.9	28.0	28.0	28.1	28.2	28.3	28.4	28.5
1030	28.7	28.7	28.8	28.9	29.0	29.1	29.1	29.2	29.4	29.4
1031	29.6	29.6	29.7	29.8	29.9	30.0	30.1	30.2	30.3	30.4
1032	30.5	30.5	30.6	30.7	30.9	31.0	31.1	31.2	31.3	31.4
1033	31.4	31.4	31.5	31.6	31.8	31.9	32.0	32.1	32.3	32.4
1034	32.3	32.3	32.4	32.5	32.7	32.9	33.0	33.1	33.2	33.3
1035	33.1	33.2	33.4	33.5	33.6	33.8	33.9	34.0	34.2	34.3

Specific gravity.	Degrees of thermometer (Fahrenheit).									
	56	57	58	59	60	61	62	63	64	65
1020	19.7	19.8	19.9	19.9	20.0	20.1	20.2	20.2	20.3	20.4
1021	20.7	20.8	20.9	20.9	21.0	21.1	21.2	21.3	21.4	21.5
1022	21.7	21.8	21.9	21.9	22.0	22.1	22.2	22.3	22.4	22.5
1023	22.7	22.8	22.8	22.9	23.0	23.1	23.2	23.3	23.4	23.5
1024	23.6	23.7	23.8	23.9	24.0	24.1	24.2	24.3	24.4	24.5
1025	24.6	24.7	24.8	24.9	25.0	25.1	25.2	25.3	25.4	25.5
1026	25.6	25.7	25.8	25.9	26.0	26.1	26.2	26.3	26.5	26.6
1027	26.6	26.7	26.8	26.9	27.0	27.1	27.3	27.4	27.5	27.6
1028	27.6	27.7	27.8	27.9	28.0	28.1	28.3	28.4	28.5	28.6
1029	28.6	28.7	28.8	28.9	29.0	29.1	29.3	29.4	29.5	29.6
1030	29.6	29.7	29.8	29.9	30.0	30.1	30.3	30.4	30.5	30.7
1031	30.5	30.6	30.8	30.9	31.0	31.2	31.3	31.4	31.5	31.7
1032	31.5	31.6	31.7	31.9	32.0	32.2	32.3	32.5	32.6	32.7
1033	32.5	32.6	32.7	32.9	33.0	33.2	33.3	33.5	33.6	33.8
1034	33.5	33.6	33.7	33.9	34.0	34.2	34.3	34.5	34.6	34.8
1035	34.5	34.6	34.7	34.9	35.0	35.2	35.3	35.5	35.6	35.8

Specific gravity.	Degrees of thermometer (Fahrenheit).									
	66	67	68	69	70	71	72	73	74	75
1020	20.5	20.6	20.7	20.0	21.0	21.1	21.2	21.3	21.5	21.6
1021	21.6	21.7	21.8	22.0	22.1	22.2	22.3	22.4	22.5	22.6
1022	22.6	22.7	22.8	23.0	23.1	23.2	23.3	23.4	23.5	23.7
1023	23.6	23.7	23.8	24.0	24.1	24.2	24.3	24.4	24.6	24.7
1024	24.6	24.7	24.9	25.0	25.1	25.2	25.3	25.5	25.6	25.7
1025	25.6	25.7	25.9	26.0	26.1	26.2	26.4	26.5	26.6	26.8
1026	26.7	26.8	27.0	27.1	27.2	27.3	27.4	27.5	27.7	27.8
1027	27.7	27.8	28.0	28.1	28.2	28.3	28.4	28.6	28.7	28.9
1028	28.7	28.8	29.0	29.1	29.2	29.4	29.5	29.7	29.8	29.9
1029	29.8	29.9	30.1	30.2	30.3	30.4	30.5	30.7	30.9	31.0
1030	30.8	30.9	31.1	31.2	31.3	31.5	31.6	31.8	31.9	32.1
1031	31.8	32.0	32.2	32.2	32.4	32.5	32.6	32.8	33.0	33.1
1032	32.9	33.0	33.2	33.3	33.4	33.6	33.7	33.9	34.0	34.2
1033	33.9	34.0	34.2	34.3	34.5	34.6	34.7	34.9	35.1	35.3
1034	34.9	35.0	35.2	35.3	35.5	35.6	35.8	36.0	36.1	36.3
1035	35.9	36.1	36.2	36.4	36.5	36.7	36.8	37.0	37.2	37.3

PLATE XXIII.

Babcock. Flask. showing Fat in Neck. (Harrington).

Estimation of the Fat.—The estimation of the fat is most conveniently accomplished with the aid of the *Babcock apparatus*. Special tubes (Plate XXIII) which have a long, graduated neck accompany the instrument. With a special pipette 17.6 c.c. of milk are measured off and introduced into one of the flasks. To this is added an equal volume of concentrated sulphuric acid (specific gravity 1.8), which should be slowly done, agitating the mixture gently by a rotary motion. The flask is placed in the centrifugal machine (counterbalanced by a flask, similarly weighted) and whirled for five minutes. After this boiling water is added to the base of the neck of the flask and the mixture centrifugated for three more minutes; boiling water is then further added until the layer of fat is well within the neck of the bottle and centrifugation continued for two to three minutes longer. The percentage of fat is finally read off directly on the neck of the bottle. If it should happen that the fat is caked in the tube, this is placed in hot water and melted, after which the reading is taken.

For human milk Leffman and Bean have modified these bottles. They are graduated so that each small division corresponds to 0.3 per cent. of fat. The sulphuric acid is of the same strength as above, but the amount of milk which is needed is only 2.92 c.c., which is measured off by means of a special pipette. This is treated with an equivalent volume of sulphuric acid, after the previous addition of 0.6 c.c. of a mixture of equal parts of concentrated HCl and amyl alcohol. Special jackets are provided into which the test bottles fit, and these can be attached to the common laboratory centrifuge used in urine work.

Estimation of Lactose.—The lactose may be estimated polarimetrically or as follows: Dilute 10 c.c. of milk to 50 c.c. with water and add dilute acetic acid carefully until all the casein has separated out. Filter and wash the precipitate with water until the total bulk of the filtrate is 100 c.c. Boil in order to remove the coagulable albumins; filter again, wash through the filter until the filtrate is again brought to 100 c.c. In this final solution determine the amount of lactose by Fehling's method (which see). 10 c.c. of Fehling's solution require 0.067 gram of lactose for the complete reduction of the copper.

Estimation of the Proteids. Boggs' Method.[1]—This is the most satisfactory for routine work and can be warmly recommended. It is based upon the precipitation of the proteids with phosphotungstic acid in hydrochloric acid solution, and in Esbach tubes.

The reagent is prepared as follows: 25 grams of phosphotungstic acid are dissolved in 125 c.c. of distilled water. To this are added concentrated hydrochloric acid 25 c.c., diluted with distilled water 100 c.c. This is essentially a 10 per cent. solution of phosphotungstic acid in 3 per cent. HCl; it is said to keep for months, when kept in a dark bottle.

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[1] Johns Hopkins Hosp. Bull., vol. xvii, October, 1906.

The Esbach tubes which are used are the common ones, reading from 1 to 7 grams pro liter; those reading to 12 gave unsatisfactory results.

The milk is diluted to 1 in 10 for human milk and 1 in 20 for cows' milk; if the proteid content is very low a dilution to 1 in 5, viz., 1 in 10 will answer.

The diluted milk is poured into the tube to the mark *U* and the reagent added to *R*, reading the bottom of the meniscus. After closing with a stopper the tube is inverted a dozen times and set aside in a vertical position for twenty-four hours. With dilutions of 1 in 10 the percentage is read off directly from the scale, while with a dilution of 1 in 20 we multiply by 2 and with one of 1 in 5 we divide by 2.

It is essential that the dilutions should be accurate. A convenient outfit consits of a 2 c.c. pipette graduated in tenths and a small standard flask of 20 c.c.

CHAPTER XIV.

THE OPSONINS.

THE term *opsonin* has been introduced by Wright and Douglas to designate certain substances present in blood serum which render various bacteria subject to phagocytosis. The organisms in question are the various staphylococci, streptococci, pneumococci, meningococci, gonococci, influenza bacilli, diphtheria and pseudodiphtheria bacilli, anthrax bacilli, tubercle, typhoid and colon bacilli, the plague bacillus, and others. For all these *normal* human blood serum contains opsonic material, but it is to be noted that with certain bacteria phagocytosis only occurs if the strains are not highly virulent. This is notably the case with streptococci and pneumococci. On the other hand, it should be borne in mind that with some organisms phagocytosis will take place in normal salt solution, in the absence of blood serum (Bacillus pyocyaneus, Bacillus subtilis, and others).

While normal opsonins are more or less thermolabile, being usually destroyed by heating for ten minutes at 60° C., the opsonins of immune sera are generally speaking more stable. Whether or not the immune opsonins are identical with the normal opsonins, as Wright claims, has not been definitely established. My own researches, in contradistinction to those of Wright and Bulloch tend to show that the normal opsonins are non-specific, while in infections results are at times obtained which suggest a certain specificity of the opsonic material.

Of the chemical nature of the opsonins nothing is known. In association with Lamar, I have shown that they are intimately associated with the euglobulin fraction of the blood albumins, but there is some reason to think that they are carried down only mechanically with this fraction and that they are not necessarily globulins themselves.

Of the structure of the opsonins also nothing is known. Hektoen suggests that they may contain a haptophoric group which unites with bacterial or other receptors, and a functional opsoniferous group which brings about such changes in the cell as may be necessary to subject it to phagocytosis.

Savtchenko and Dean view immune opsonins as amboceptors, but have not furnished a satisfactory basis for such an assumption. Greig-Smith regards the immune opsonins and agglutinins as identical, and looks upon the process of opsonification as the first phase of agglutination, but also, I think, without an adequate basis.

Opsonins occur in all classes of vertebrates, and it is noteworthy that the serum of different animals (fish, frog, turtle, chicken, guinea-pig, rabbit, calf, sheep, pig, dog, cat, etc.) is capable of activating various microörganisms for phagocytosis by leukocytes of animals of different species.

Besides bacteria other cells can also be opsonified. Barrath has noted the presence of opsonins in small amount for red cells in normal serum (hemopsonins). Hektoen obtained similar results and also showed that blastomycetes from human lesions become surrounded by masses of leukocytes in the presence of normal human and dog serum. Preliminary observation (according to Hektoen) further indicates that phagocytosis of trypanosomes also is dependent upon opsonification. Savtchenko and Melkich obtained pronounced phagocytosis of the spirochæte of relapsing fever with serum of convalescent patients.

Clinical interest centres in Wright's observation that in certain bacterial infections the opsonins are frequently diminished, and that it is possible by means of bacterial vaccines to raise the opsonic value and thereby to increase the patient's resistance to the invading microorganisms. As a matter of fact it has now been satisfactorily established that the bacterial vaccines represent a most important addition to our therapeutic armamentarium and that it is possible in many cases to cause either a cure or an improvement in the patient's condition by means of such vaccines, where with older methods of treatment no result at all or only very slow improvement could be expected. This is notably the case in the more chronic bacterial infections, such as acne, sycosis, furunculosis, various types of tuberculosis (notably the so-called surgical forms), endocarditis, bacterial arthritis, unresolved pneumonia, empyema, the most diverse wound infections, etc.

The basis in the treatment of these various conditions, according to Wright, is the opsonic index, viz., the opsonic value of the infected individual as compared with the normal. According to his teachings the injection of a dose of vaccine is followed by a decrease of the opsonins (the negative phase), which is of variable degree and duration depending upon the amount injected. This is followed by an increase (the positive phase), coincidently with which there is a corresponding improvement in the patient's condition. The idea is to so gauge and interspace the different doses that a negative phase is obviated as far as possible and a "high tide" of increased opsonic content secured.

While a low opsonic value is the rule in the more chronic cases, and especially in connection with the more localized infections, high indices may be observed in acute cases with active systemic manifestations, and frequently alternate with low values.

Deviations from the normal may at times be of distinct diagnostic value when they affect a particular microörganism, and Wright cites a number of examples to emphasize this point. I cite some of his more important deductions in the diagnosis of tuberculous infection:

1. Conclusions which can be arrived at when we have at disposal the results of a series of measurements (opsonic determinations):

(*a*) When a series of measurements of the opsonic power of the blood reveals a persistingly low opsonic power with respect to the tubercle bacillus, it may be inferred, in the cases where there is evidence of a localized bacterial infection which suggests tuberculosis, that the infection in question is tuberculous in character.

(*b*) When repeated examination reveals a persistingly normal opsonic power with respect to the tubercle bacillus, the diagnosis of tubercle may with probability be excluded.

(*c*) When there is revealed by a series of blood examinations a constantly fluctuating opsonic index the presence of active tuberculosis may be inferred.

2. Conclusions which may be arrived at where we have at disposal the result of an isolated blood examination:

(*a*) When an isolated blood examination reveals that the tuberculo-opsonic power of the blood is low, we may—according as we have evidence of a localized bacterial infection or of constitutional disturbance—infer with probability that we are dealing with tuberculosis—in the former case with a localized tuberculous infection, and in the latter with an active systemic infection.

(*b*) When an isolated blood examination reveals that the tuberculo-opsonic power of the blood is high, we may infer that we have to deal with a systemic tuberculous infection which is active, or has recently been active.

(*c*) When the tuberculo-opsonic power is found normal or nearly normal, while there are symptoms which suggest tuberculosis, we are not warranted, apart from the further test described below, in arriving at a positive or a negative diagnosis.

The further criterion to which reference has been made in the preceding paragraph is the following:

When a serum is found to retain in any considerable measure, after it has been heated to 60° C. for ten minutes, its power of inciting phagocytosis we may conclude that "incitor elements" (immune opsonins) have been elaborated in the organism either in response to auto-inoculations occurring spontaneously in the course of tuberculous infection, or, as the case may be, under the artificial stimulus supplied by the inoculation of tubercle vaccine.

The above considerations apply also in the case of other bacterial infections, and in the examination of exudates as well.

In a general way my own observations bear out the correctness of Wright's diagnostic inferences, but I am inclined to attach importance only to the results of repeated examinations and to pronounced deviations from the normal variations, viz., 0.8 to 1.2. Single observations are of relatively little importance, and purely localized infections without systemic symptoms may show no deviation from the normal whatever. Positive results are thus only of value, while normal values do not exclude the existence of infection.

As regards the necessity of controlling bacterial inoculation by the opsonic index, my own observations and those of some of my colleagues tend to show that the acts on this subject are by no means closed. If attempts at progressive active immunization are to be made, an index of some kind is certainly desirable, aside from purely clinical symptoms, and the opsonic index in these cases is in my opinion unquestionably of value. If we see, as the result of repeated injections, that the phagocytic power of the patient is rapidly being diminished and finally practically held in abeyance, there can be no doubt to my mind that immunization in such a case is not being carried out to best advantage. The index in such cases would certainly be of value. Then, again, if we find that a single inoculation in a given case invariably causes a marked drop in the phagocytic power, while smaller doses do not bring about this result, it is clear that here also the determination of the index would be of value. I should hence advocate its use in therapeutic work, especially in cases showing active disease, and notably so in children in whom a marked depression of the phagocytic power may be caused by using vaccine in the doses recommended for adults.

In markedly chronic cases, on the other hand, in which the opsonic index shows but little variation from the normal, bacterial vaccines may safely be used in small doses and at intervals of from eight to ten days with but little control by the index.

An opsonic immunity, in the sense of a continued high index as the result of immunization, does not exist. In the majority of cases the injection of a suitable dose of vaccine causes no negative phase which would not readily be explained by unavoidable errors of technique; after several days there is then a rise of a few tenths and after that a return to near the normal line. Continued high values are very rarely seen; sooner or later there is a return to normal, even though improvement continues.

TECHNIQUE.

Principle.—Wright's method is based upon the comparison of the number of organisms taken up by a given number of leukocytes under the opsonifying effect of the patient's serum, with the corresponding number observed in the case of a normal control serum, the latter value being placed as 1.

Example.—Supposing that with the patient's serum the average number of organisms pro cell (the phagocytic index) was 5 and with the normal serum 10, then from the equation $10:1::5:x$, it would follow that the opsonic index was 0.5.

I have pointed out at another place that Wright's method is open to certain fallacies, and that more accurate results can be obtained by estimating the percentage of phagocyting leukocytes. By comparing the figures thus obtained with the figure corresponding to a specimen of normal blood serum, terming the latter value 1, an index is obtained which is directly comparable to Wright's index. As the percentage of phagocyting cells is to a certain extent dependent on the number of organisms present, it is advantageous to work with an emulsion which with normal serum will give a percentage of about 50; this will allow for an increase of the index in the patient's blood to about 2, which is sufficient for all practical purposes. If higher values are to be expressed it is necessary to dilute the serum (both normal and that of the patient) in the proportion of 1 to 10, 1 to 20, etc., with saline solution and to proceed upon the same principle.

Example.—With the patient's serum 80 per cent. of the cells were found to be phagocyting and with normal serum only 50. The index would be obtained according to the equation $50:1::80:x$, and would accordingly be 1.6.

If for any reason Wright's *bacillary index* is to be used, I should recommend that the percentage index be calculated at the same time; it will be found a useful check upon the former and readily shows up errors that may have been made in counting, depending on clumping of the organism. Under favorable conditions both will agree to the second decimal. If both are normal, above or below normal, Wright's index may be regarded as giving more or less correct values, but if they differ, the one being high and the other low, the percentage value should be accepted in lieu of the other.

To obtain the best idea of the opsonic content of the blood, estimations should be made not only with the concentrated serum, but also with dilutions, and I would recommend that 1 to 20 and 1 to 40 be accepted as standards; in that case the emulsion of organisms should be made rather dense so as to give values between 50 and 100 for concentrated normal blood. The best indeed would be if all opsonic workers were to accept a definite standard in this respect, as results would then be more directly comparable.

Method.—The material necessary for an opsonic estimation is the following:

The patient's serum.
Normal control serum.
Washed corpuscles.
The bacterial emulsion.

1. *Preparation of the Patient's Serum.*—A small amount of blood (about 6 or 8 drops) is collected from a puncture of the ear by means of a little pipette (Plate XXIV, Fig. *a*) and immediately transferred to a small glass tube having a diameter of about one-quarter of an inch (Plate XXIV, Fig. *b*), which is then tightly corked. The blood is allowed to clot, the coagulum separated from the walls of the tube, and the specimen centrifugalized (water or electrical centrifuge) until the corpuscles have been packed down and thus separated from the serum.

Wright recommends the collection of the blood in special capsules (Plate XXIV, Fig. *c*), which are then sealed in a flame. He obtains the blood by puncturing the thumb near the root of the nail, after having previously allowed the arm to hang down and then applying some constriction hehind the distal joint (tape, rubber tubing). The puncture is made with a fine glass needle obtained by drawing out a piece of glass tubing in the flame of a small burner. When the bent capillary of the capsule is held to the exuding blood it enters by capillary attraction. On warming the body of the capsule the blood rises into it, when both ends are sealed.

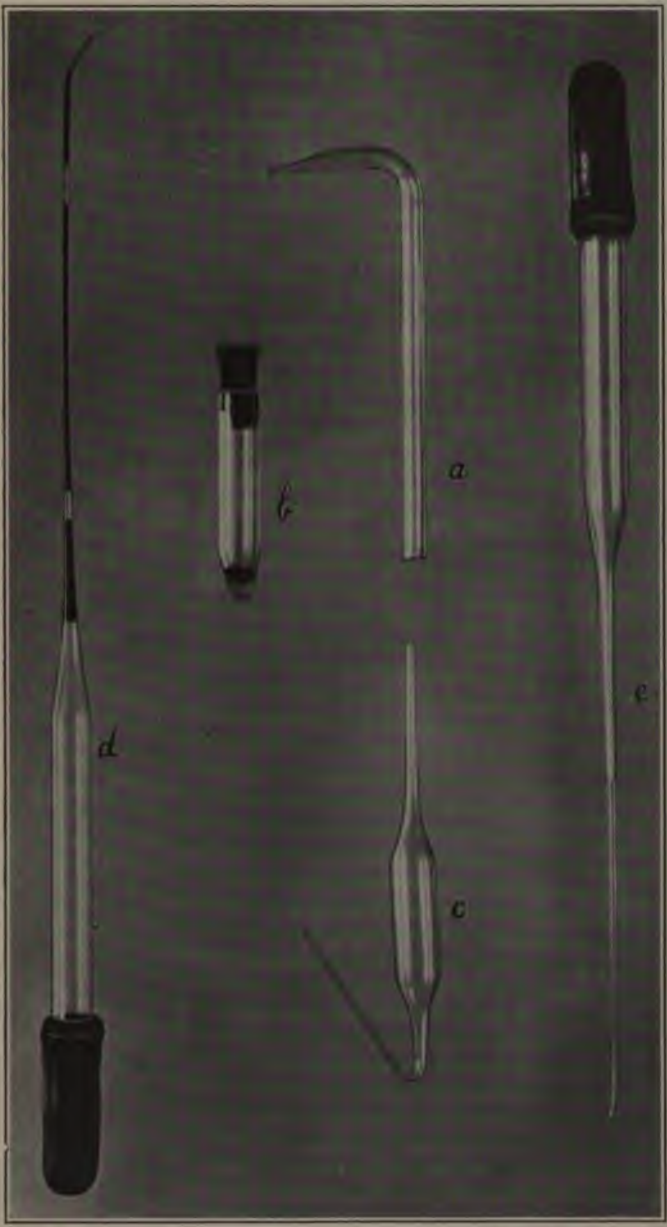
In my experience Wright's procedure offers no material advantages over the simpler method which I employ myself. The serum should not be older than twenty-four hours.

2. *Preparation of Normal Control Serum.*—This is collected in the same manner as the patient's serum and separated from the corpuscles by centrifugatin. It is preferable to pool three or four normal sera, viz., to mix equal quantities from three or four individuals. If, however, the serum of one single person (of the experimenter, for example) has been thoroughly studied and always found normal, this single serum may suffice for all ordinary purposes. Women during menstruation, hard smokers, and individuals who are pale and below weight, even if otherwise healthy, should not be taken as control, nor included in a pool. Occasionally apparently normal individuals are also met with, who habitually have a higher opsonic content than normal, and such must of course also be excluded. The process of digestion further tends to increase the opsonic content of the blood, so that it is advisable to take the blood of the patient and the pool approximately at the same time of the day.

As with the patient's blood, the control serum should not be older than twenty-four hours; in my own work I use no blood that is older than twelve hours.

3. *Preparation of Washed Corpuscles.*—The blood is most conveniently collected from the ear and received in a tube containing either 1.5 per cent. of sodium citrate solution or 1.2 per cent. saline, containing 0.1 per cent. ammonium oxalate to prevent clotting. The amount will depend upon the number of specimens that are to be pre-

PLATE XXIV.



Apparatus for Opsonic Work.

(*a*) Pipette for collecting blood; (*b*) Tube to receive blood for separation of rum; (*c*) Wright blood capsule; (*d*) Blood pipette charged with corpuscles, rum, and bacterial emulsion; (*e*) Same, in solid column, ready for incubation.

pared; 1 c.c. of blood is sufficient for at least a dozen mounts. Small test-tubes of 5 c.c. capacity are very convenient. (The anticlotting fluid should be watched and the supply renewed when it becomes turbid.) Clots must be avoided and the specimen promptly discarded if the slightest coagulum has formed. This will rarely occur with reasonably fresh anticlotting fluid. The tubes are centrifugalized until the corpuscles have been well packed down and an opaque little film (of leukocytes) can be made out on top of the red cells. The supernatant fluid is then pipetted off and replaced with 1.2 per cent. saline; the washing is repeated once more and after the corpuscles have been again packed down the fluid is carefully withdrawn (the last traces with a capillary pipette). Wright then uses the superficial layer of corpuscles only, as this is especially rich in leukocytes (the leukocytic cream). In my laboratory we shake up the cells thoroughly and find that we obtain a sufficient number of leukocytes in this way also.

The washed corpuscles should not be kept longer than five or six hours.

4. *Preparation of the Bacterial Emulsions.*—Perfectly uniform bacterial emulsions cannot be secured as a matter of routine; some clumps, if only of a few organisms, are practically unavoidable. For this reason I prefer the percentage index to the bacillary index, as it is not subject to errors arising from this source.

With some organisms, an emulsion of a fair degree of uniformity is more readily obtained than with others; with the tubercle bacillus especially it is very difficult.

Emulsions of Cocci.—Staphylococci and streptococci may be grown on plain agar, while gonococci, pneumococci, and meningococci are cultivated on blood agar or hydrocele agar. Small tubes, such as the one pictured on Plate XXIV, are charged with a little saline solution (0.85 to 1.2 per cent.). A bit of the culture is removed with a platinum loop and *very gently* rubbed against the wall of the tube, at the surface of the liquid; this must be done with a light hand, and slowly. When the fluid has become turbid it is centrifugalized for a few minutes so as to remove clumps as far as possible and to acquire the proper degree of thickness. This point can only be learned by experience; trial tubes (see below) should be filled and specimens mounted from emulsions, showing various degrees of turbidity. Wright obtains the best result if four or five cocci are found pro cell, while with the percentage method I aim at a thinner emulsion, viz., one furnishing about 50 per cent. of phagocyting cells. Small glass capsules may be prepared containing emulsions of barium sulphate of varying degrees of turbidity, and corresponding to standard emulsions of the various organisms; these are convenient in determining how far the centrifugation is to be carried.

It has been recommended that the cultures should always be fresh and not more than twenty-four hours old. This is not necessary with all organisms. Knorr has shown that the same degree of phagocytosis is obtained with cultures of the staphylococcus more than a month old, as with young cultures, and in my laboratory we have worked successfully with one and the same emulsion preserved with a few drops of chloroform for a couple of months.

Emulsions of Colon and Typhoid Bacilli.—Wright recommends the use of cultures only four hours old. With older cultures the spherulation of the organisms is such that approximate results only can be obtained. The percentage method with these organisms is certainly far superior to Wright's method. The emulsions are prepared in the same way as directed for the cocci.

Emulsions of the Tubercle Bacillus.—Cole has obtained the best results by starting with living cultures of the tubercle bacillus on glycerin agar which are killed by exposure to sunlight for twenty-four hours. Some of the material is then scraped off, ground up in an agar mortar with 1.5 per cent. saline, and centrifugalized to remove clumps. If contamination is guarded against the supernatant emulsion can be used for at least a month.

I have not had an opportunity to use emulsions prepared in this manner, and am familiar only with material prepared from dried and ground-up dead bacilli. A small amount of these is placed in an agar mortar and ground up with 1.5 per cent. saline solution, which is *slowly* added drop by drop. The emulsion is centrifugalized free from coarse clumps, but always contains smaller ones which are practically impossible to remove. I have worked with extracted and non-extracted bacilli, with 0.1 and 1.5 per cent. saline, with heated and unheated bacilli, but have not yet seen an emulsion of the tubercle bacillus that was free from clumps.

Wright recommends that the emulsion should be of such thickness that one or two organisms are found on an average for each cell, while I aim at approximately 50 per cent. of phagocyting cells.

5. *Charging the Pipette.*—Having prepared the patient's serum, normal control serum, washed corpuscles, and bacterial emulsion, these tubes are conveniently placed in a dishful of sand covered with a piece of white filter paper, perforated to receive the tubes and marked accordingly.

Mixing pipettes are prepared from glass tubing having an inside diameter of approximately 6 mm. (¼ inch). To this end pieces of tubing are cut, measuring about 15 cm. (6 inches) in length, and drawn out in the flame of a Bunsen burner, so that capillary stems are obtained about 15 to 18 cm. (6 to 7 inches) long, with an approximate diameter of 1 mm., or a trifle less. The ends are cut off square with a fine file. The tubes are marked about ¾ to 1 inch from the ends with a glass pencil and provided before use with a rubber nipple (ordinary medicine-dropper nipples). (See Plate XXIV.) One volume of corpuscles is then drawn up to the mark, followed by one volume of serum and one of bacterial emulsion, the three portions being separated from one another by little bubbles of air. The contents of the tube are next blown out upon a slide, well mixed, drawn up and blown out repeatedly, and finally drawn up in a solid column, holding the pipette almost vertically to avoid bubbles of air. The end of the capillary stem is sealed in the flame of a Bunsen burner or an alcohol lamp and the tube incubated at 37° C. for fifteen minutes.

6. *The Slide Mount and Staining.*—After incubation the end of the tube is pinched off, a large drop mounted upon a clean slide, stirred with the end of the tube, and a spread made with a second slide as in ordinary blood work, only a little thicker and using no force whatsoever. (See Fig. 17.) After drying in the air the specimens (excepting tubercle mounts) are stained without previous fixation, either with a 1 per cent. aqueous solution of methylene blue or with some polychrome dye like Jenner's, Hastings', Giemsa's, etc. I find the aqueous methylene blue especially convenient, as it largely removes the hemoglobin from the red cells; thicker specimens can thus be prepared and there will consequently be more leukocytes. If the leukocytic cream is used, thin preparations can be made and stained with polychrome dyes; the resulting pictures are very pretty.

Tubercle specimens are fixed by immersion for ten minutes in a saturated aqueous solution of mercuric chloride. They are then washed off in water, stained with steaming carbol-fuchsin solution (Czaplewsky's formula, p. 345, foot note 5), washed in water, decolorized in 2 per cent. sulphuric acid, washed, immersed for a few seconds in 0.1 per cent. sodium carbonate solution, washed again, counterstained for one minute with 1 per cent. aqueous methylene blue, washed once more, and set up to dry.

7. *Counting.*—To obtain the bacillary index a series of 50 or more polynuclear leukocytes are examined and the number of organisms in each noted; the average for one represents the phagocytic index.

To obtain the percentage index it is only necessary to note the number of phagocyting cells in the series and to work out the percentage. The opsonic index in either case is calculated by dividing the patient's value by the normal control, as already described.

To obtain reliable counts much practice is necessary, and every one will have to work out his own personal equation in deciding what organisms are to be counted in or out, when lying in the margin of the cell, whether a certain cell is to be excluded because it contains too many organisms to be counted, how many negative cells are to be thrown out to balance an eliminated positive cell, etc. For this reason the counts between two individuals will often vary considerably, unless a very large number of cells has been gone over, while for the same person comparative counts will agree much more closely.

LITERATURE.—Wright and Douglas, Proc. Royal Soc., 1903, vol. lxii, p. 357; ibid., 1904, vol. lxxiii, p. 128; ibid., p. 135; ibid., p. 147. Wright and Read, ibid., 1906, vol. lxxvii, p. 194, and ibid., p. 211. Hektoen and Rüdiger, Journ. of Infect. Diseases, 1905, vol. ii, p. 128. Hektoen, Phagocytosis and Opsonins, Journ. Amer. Med. Assoc., May 12, 1906. Simon and Lamar, Johns Hopkins Hospital Bull., 1906, vol. xvii, p. 27. Simon, Lamar, and Bispham, Journ. Exper. Med., 1906, vol. viii, p. 651. Simon, Journ. Amer. Med. Assoc., Jan. 12, 1907, p. 138. Potter, Ditman, and Bradley, ibid., Nov. 24 and Dec. 1, 1906. Knorr, ibid., April 13, 1907, p. 1256. Ross, "The Opsonic Theory and its Application to Medicine and Surgery," Brit. Med. Journ., 1906. Bulloch, London Practitioner, Dec., 1905. Wright, Lancet, Dec. 2 and Dec. 9, 1905. Crace-Calvert, "Opsonins and the Opsonic Index and their Practical Value in the Treatment of Disease," Lancet, Feb. 2, 1907.

APPENDIX.

A.

PREPARATION OF CULTURE MEDIA.

Nutrient Bouillon.—Dissolve 6 to 8 grams of Liebig's beef extract together with 5 grams of sodium chloride and 10 grams of Witte peptone in about 100 c.c. of water by the aid of heat, stirring with a glass rod. Render the solution faintly alkaline to litmus (red paper should turn faintly blue, while the blue paper remains unchanged) by adding a fairly concentrated solution of sodium carbonate drop by drop. Or titrate 10 c.c. with $\frac{1}{10}$ normal alkali, using phenolphthalein as an indicator, to the point of the first pink which persists; estimate the corresponding amount of normal alkali which must accordingly be added to the remaining bulk of the fluid; add this and dilute to 1000 c.c.

Example.—10 c.c. required the addition of 10 c.c. of $\frac{n}{10}$ normal alkali. There remain 90 c.c. of bouillon; for each 10 c.c. in this, viz., 9, it is necessary to add 10 c.c. $\frac{n}{10}$ alkali; so in this case 90 c.c. Instead of using so much of the $\frac{n}{10}$ solution it is convenient to use 9 c.c. of the full-strength normal solution. This, however, is not necessary; the $\frac{n}{10}$ normal, in the amount mentioned, can be used, if *it* only is available.

If by any chance too much alkali has been added, use very dilute hydrochloric acid to return to the neutral point.

In any event test the final reaction with litmus paper and see to it that the reaction is slightly but distinctly alkaline while blue litmus paper remains unchanged. Then filter into a liter flask, plug the mouth with cotton, and sterilize for one hour in the steam sterilizer. After that tubes are filled to the desired height (1½ to 2 inches) and again sterilized.

Glucose Bouillon.—This is nutrient bouillon to which 1 to 2 per cent. of glucose has been added.

Lactose Bouillon.—Nutrient bouillon, containing 1 to 2 per cent. of lactose.

Other carbohydrate bouillons contain corresponding amounts of material.

Nutrient Gelatin.—6 to 8 grams of Liebig's beef extract, 5 grams of sodium chloride, and 10 grams of Witte peptone are dissolved in a liter of water, as in the preparation of nutrient bouillon. To this solution 100 to 150 grams of gelatin are added, the latter broken up into small pieces. The mixture is boiled in an agate saucepan, stirring frequently so as not to burn the gelatin at the bottom. It is then neutralized as described above (preparation of nutrient bouillon), and clarified by the addition of the white of an egg beaten up in 50 c.c. of water. Before this is added the solution should be allowed to cool to 60° C. After this the boiling is continued for fifteen minutes, allowance being made for evaporation by the addition of a little water from time to time. The solution is then filtered. To this end no hot-water funnel or other artificial contrivance is necessary. The essential requisite is that the gelatin is in solution and has been actually boiling. The filter is wetted thoroughly before; if the first 4 c.c. should pass through turbid they are passed back. If the filtration should cease, the material in the funnel must be further boiled and the filtration continued thereafter.

The filtrate is received in a flask, plugged with cotton, and sterilized on three consecutive days in the Arnold sterilizer for fifteen to twenty minutes daily. Tubes, however, can be charged on the first day and the sterilization carried on in these.

Nutrient Agar.—This consists of nutrient bouillon, containg 1 to 1.5 per cent. of agar. The agar (10 to 15 grams) is cut into very small pieces and placed for twenty-four to forty-eight hours in 600 c.c. of water containing the 5 grams of salt required for the liter of bouillon. In the mean time the 6 to 8 grams of Liebig's beef extract and 10 grams of peptone are dissolved in 400 c.c. of water, neutralized as described (see Nutrient Bouillon), and sterilized. After soaking as indicated, the agar-salt mixture and the neutralized beef-peptone solution are poured together in an agate saucepan and the depth of the liquid measured; 300 c.c. of water are then added to allow for evaporation during the two hours and a half of active boiling which must follow. During this process the liquid must not fall below its original bulk. The white of an egg beaten up in 50 c.c. of water is then added (the liquid should be previously allowed to cool to 60° C. by setting the pan in a vessel with cold water), after which the boiling is continued actively for half an hour longer, when the agar is filtered through a previously prepared filter which has been well wetted. If the agar is well in solution the liter will pass through in little more then half an hour. If filtration should stop, the material must be boiled again and a new filter prepared. The agar can be filtered into tubes the same day or kept in a plugged flask; in either case it must be sterilized for three consecutive days in the steam sterilizer for fifteen to twenty minutes daily.

If agar slants are to be prepared, care must be taken not to fill the tubes too high. After their final sterilization they are laid down, slightly elevated at the open end, so that the agar forms a long slant; in this position they remain for some hours (over night).

Glycerin Agar.—This is nutrient agar containing 6 to 8 per cent. of glycerin. This is added after filtration and before sterilization.

Glucose Agar.—This is nutrient agar containing 1 to 2 per cent. of glucose. The glucose is conveniently dissolved in the beef extract-peptone portion.

Other carbohydrate agars contain corresponding amounts of material.

Litmus Agar.—This is ordinary agar which has been colored by the addition of a 5 per cent. solution of purified litmus; the agar should show a bluish color.

Litmus-Carbohydrate Agar.—Litmus agar containing 1 per cent. of one of the various carbohydrates—dextrose, lactose, mannite, etc.

Hydrocele Agar (Cushing).—The fluid (hydrocele or ascitic) is obtained sterile, the locality of puncture being carefully sterilized by modern surgical methods, the sterile trocar covered at its external end with sterile gauze, so as not to be infected by the operator's hand, and the fluid collected in sterile flasks, the sterile stoppers being then replaced. When collected in this way it rarely becomes contaminated and may often be kept for months before using. This fluid is mixed with ordinary nutrient agar. A number of common agar slants are placed in the autoclave for five minutes. This liquefies the agar and at the same time thoroughly sterilizes the tubes and cotton stoppers. The slants are then put in a water bath at 55° C., so as not to coagulate the albumin when mixed with the agar. The stopper having been removed from a small flask of hydrocele fluid, the top of the flask is flamed and the albuminous fluid then poured into an agar tube (the top of which has also been flamed) in the proportion of a little more than 1 to 2. It is well to have as much of the hydrocele fluid as the future solidity of the medium will allow. Ordinary agar will allow not quite equal parts of the two. The stopper is then returned to the agar tube, which is immediately slanted. On these slants gonococci grow most abundantly in or near the liquid which is squeezed out of the medium and collects at the bottom of the tube. Some cultures will maintain a vigorous growth after numerous transplantations, while others again grow only two or three times, or indeed once only.

Blood Agar.—Agar tubes are melted, as just described, and then placed in a water bath at 50° C. To each tube approximately one-half of a cubic centimeter of human blood is added. Agar and blood are well mixed and the tubes immediately slanted. Before use they should be incubated for twenty-four hours to see that they are sterile. The necessary blood is obtained by aspirating a vein with a sterile syringe, containing a little 1 per cent. sodium citrate to prevent coagulation, or it may be collected in a sterile glass pipette from the ear under antiseptic precautions.

Neutral Red Agar.—Agar 2 per cent., grape sugar 0.3 per cent., neutral red solution 1 c.c. (saturated watery solution of Ehrlich's neutral red). Mix; sterilize.

Dunham's Solution.—This is common nutrient bouillon without the addition of Liebig's beef extract. Its reaction is neutral or slightly alkaline *per se*, and need hence not be corrected. The solution is filtered, tubes filled and sterilized, as in the case of bouillon.

Litmus Milk.—Fresh milk which has been freed from cream, as far as possible, is treated with tincture of litmus until it presents a distinct blue color. Tubes are filled with this and sterilized on two successive days for an hour at a time.

Litmus Whey.—To 500 c.c. of milk add 10 to 12 c.c. $\frac{n}{1}$ solution HCl to precipitate the casein. Neutralize with soda solution. Boil one to two hours. Let the precipitate fall to the bottom. Take 100 c.c. of fluid and add 5 c.c. litmus solution. Place in tubes; sterilize for from two to three hours at 100° C.

Potato Slant.—Large potatoes are selected. They are thoroughly scrubbed in running water and cylinders forced out with a large cork borer. They are cut square at the ends and then obliquely into two parts. The resultant wedges are kept over night in running water and the next day are placed in sterile tubes. The potato tubes are steamed for one hour.

Loeffler's Blood Serum.—3 parts of ox-blood serum are mixed with 1 part of nutrient bouillon containing 1 per cent. of glucose. Tubes are filled with this mixture and coagulated in a slanting position in the drying oven at a temperature a little above 90° C. It is important to raise the temperature to this point quite gradually. Here they remain until the slants are quite firm, after which they are sterilized in the steam sterilizer at 100° C. for fifteen minutes at a time, on three consecutive days.

The blood necessary for the preparation of the medium is procured at a slaughter-house. Care should be taken that it flows directly from the cut vessel into a suitable receptacle, which has been previously sterilized. Museum jars are convenient for this purpose. After coagulation has set in the coagulum is carefully separated from the walls of the vessel with a sterile glass rod and the blood kept in a cool place (ice-box). The serum which separates out is pipetted off with sterile pipette and placed in sterilized and plugged cylinders or test-tubes until required.

Two gallons of blood will approximately yield from 500 to 700 c.c. of serum.

Hiss' Serum-water Media.—The serum water is composed of beef serum 1 part and distilled water 2 or 3 parts. To this 1 per cent. of a 5 per cent. solution of highly purified litmus is added. The medium is heated for a few moments to 100° C., when 1 per cent. of either dextrose, lactose, maltose, saccharose, raffinose, dextrin, glycogen, inulin, mannite, or dulcite is added. Tubes are then filtered and sterilized on three consecutive days by steam at 100° C. for fifteen minutes at a time.

The Drigalski-Conradi Medium. 1. AGAR PREPARATION.—To 3 pounds of finely cut beef add 2 liters of water. Allow it to stand till next day. The expressed meat juice is boiled for one hour and filtered. Add 20 grms. of Witte peptone, 20 grms. nutrose, 10 grms. NaCl; boil one hour, now add 70 grms. bar agar, then boil three hours (or one hour in autoclave), render slightly alkaline (indicator litmus paper). Filter; boil half an hour.

2. LITMUS SOLUTION.—Litmus solution (Kubel and Tieman) 260 c.c., boil for ten minutes; add milk sugar (chemically pure) 30 grms. Boil fifteen minutes.

3. Add the hot litmus-milk-sugar solution to the liquid agar solution cooled to 60° C. Shake well. Render it again faintly alkaline. The color of the froth is a good indicator. Add then 2 c.c. of a hot sterile solution of 10 per cent. water-free soda; further add 20 c.c. of a freshly prepared solution of 0.1 grm. crystal-violet B. (Höchst) in 100 c.c. of warm water (distilled).

One has now a meat-water peptone-nutrose agar with 13 per cent. litmus and 0.01 per mille crystal violet. This can be poured directly into plates and the remainder kept in 200 c.c. flasks.

The Malachite-green Enriching Method of Lentz.—The proper preparation to use is malachite green (crystal) (Höchst); dilution 1 to 22,000. Preparation: 3 pounds of lean beef, finely divided, are macerated with 2 liters of water for sixteen hours. The extract is expressed, boiled for half an hour, filtered, then 3 per cent. agar added and boiled for three hours; then add 1 per cent. peptone, 0.5 per cent. NaCl, and 1 per cent. nutrose (this may be omitted). The mixture is brought to the litmus-neutral point by soda solution, boiled one hour, and filtered through linen. The reaction of the finished agar is sometimes distinctly acid. It is filtered into small flasks of 100 to 200 c.c.

Before the addition of the malachite green the hot agar is tested with neutral litmus paper and so far alkalinized with sterile soda solution until the strip is distinctly red-violet. This reaction point corresponds in agar, without nutrose, to an alkalescent degree of 1.8 per cent. normal soda below the phenolphthalein-neutral point; if the agar contains nutrose, which remains neutral toward litmus, then the alkaline reaction corresponds to 3.5 per cent. normal soda solution below the phenolphthalein point.

To 100 c.c. of the hot agar 1 c.c. of a 1 to 220 solution of malachite green (the solution is stable for ten days) is added; the agar thus contains 1 in 22,000. With this concentration of malachite green (crystal) the growth of the usual kinds of *B. coli*, as well as many alkali-forming organisms, is greatly diminshed and practically prevented.

The *B. typhosus* growth is also diminished, but only so far that after twenty-four hours the colonies can be recognized with the naked eye; they are then the size of a particle of sand, while, after a longer period in the incubator, in two to four days, larger, stronger colonies appear which color the agar yellow.

The finished agar is poured at once into Petri dishes in layers 2 mm. thick.[1]

B.

OUTLINE OF A COURSE IN CLINICAL LABORATORY METHODS.

In response to numerous requests I have arranged below a program of practical instruction in the clinical laboratory. This is based essentially upon the work done in my private courses which I have conducted for a number of years for postgraduates, and may have to be modified more or less to meet special requirements. The students who come to me for instruction are mostly general practitioners, and for their special needs this program has been arranged. The practical work is supplemented by lectures of a more or less formal and comprehensive character, as indicated below. The exercises have been collectively arranged so as to correspond to the general topics: Blood, Gastric Juice, Feces, Sputum, etc. This routine may, however, be interrupted, as special material becomes available, which cannot be advantageously preserved and should hence be examined at once.

I. **Lectures**. *A*. **Blood.**—1. General technique: the morphology of the blood, studied in the whet specimen; classification of the leukocytes, as seen in the whet specimen; percentage values; general account of variations in disease.

2. The chemistry of the aniline dyes: structure; classification; formation of neutral dyes; meaning of the terms neutrophilic, basophilic, oxyphilic, monochromatophilic, polychromatophilic, etc. Classifica-

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[1] The plates are allowed to remain open until all the steam has evaporated and the agar is stiff. It is essential that the surface of the plates should be quite dry and firm. Contamination by air organisms does not occur on account of the aniline dye present in the culture media.

tion of the leukocytes upon the basis of their behavior toward aniline dyes.

3. Preparation of stains and methods of staining: Jenner's, Giemsa's, Hastings', Goldhorn's, Ehrlich's stain.

4. Leukocytosis: significance of variations in the absolute number and of the relative percentages; neutrophilic hyperleukocytosis and the septic factor; eosinophilia; lymphocytosis; large mononucleosis; increase of mast-cells; leucopenia.

5. Origin and interrelation of the various leukocytes: myelocytes; metamyelocytes; meaning of the polymorphism of the nucleus and actual polynucleosis. Arneth's findings.

6. Leukemia.

7. The red corpuscles: poikilocytosis, anisocytosis; staining properties; granular degeneration; polychromatophilia; origin of the normocytes; normoblasts and megaloblasts; relation to leukocytes.

8. Clinical variations in the number of the red cells and the amount of hemoglobin; color index.

9. Pernicious anemia.

10. The hemocytometer and hemoglobinometer.

11. The bacteriological examination of the blood; technique and clinical indications; typhoid fever, pneumonia, pyogenic septicemia.

12. The serology of the blood: meaning of the term antigen, antibody, immunization, vaccination. Classification of the antibodies. Discussion of the formation of antitoxins, cytolysins, bacteriolysins, hemolysins, precipitins, coagulins, agglutinins, and antiferments.

13. The opsonins.

14. Malaria: life cycle of the malarial organism; asexual and sexual reproduction; methods of staining and general technique. Trypanosomiasis; relapsing fever; Kala-azar; filariasis.

B. **Gastric Juice.**—1. The gastric juice; secretion; chemical composition; test meals and the *rationale* of their employment; free hydrochloric acid and combined hydrochloric acid; euchlorhydria, hypochlorhydria; anachlorhydria and their clinical significance.

2. Analysis of the acid factors of the gastric juice: meaning of the terms normal solution, decinormal solution, indicators; technique.

3. The organic acids of the stomach contents: their origin and clinical significance; analytical methods.

4. The gastric ferments: their specific action; clinical significance of quantitative variations; analytical methods.

5. The present status of our knowledge of proteolytic digestion: concept of the terms albumose, peptone, polypeptid, etc.; proteid synthesis.

6. The microscopic constituents of the stomach contents: alimentary detritus, yeast, sarcinæ, Boas-Oppler bacillus, protozoa, tumor particles; technique.

7. The significance of the presence of blood in the stomach contents: tests for occult bleeding.

C. **The Feces.**—1. The chemistry and microscopy of normal feces; technique.

2. Animal parasites occurring in the intestinal tract and their clinical significance. Methods of examination.

3. The bacteriology of the feces.

4. The significance of the presence of blood in the feces: occult bleeding; technique.

D. **The Sputum.**—1. General account of information to be derived from a microscopic study of the sputum, with special reference to tuberculosis, pneumonia, influenza, asthma, bronchiectasis, abscess of the lung, gangrene of the lung; general technique.

2. The tubercle bacillus and related organisms; special methods.

E. **Exudates.**—1. The bacteriology of tonsillar exudates: diphtheria, tonsillitis, stomatitis, Vincent's angina; pharyngomycosis leptothricia; technique.

2. The pus in gonorrhea: the gonococcus; the pseudogonococci; pus eosinophilia.

3. Syphilis and the Spirochæte pallida.

4. The cytological and bacteriological study of pleural and peritoneal exudates.

5. The cytological and bacteriological study of cerebrospinal fluid.

F. **The Urine.**—1. General chemical study of the urine and discussion of its relative importance in the diagnosis of various pathological conditions.

2. The urinary constituents: chlorides, phosphates, sulphates.

3. Urea and nitrogenous metabolism.

4. Metabolic anomalies: lithuria, oxaluria, cystinuria, diaminuria, alkaptonuria.

5. Metabolic anomalies: diabetes and carbohydrate metabolism.

6. Albuminuria and its clinical significance.

7. The various pigments and chromogens which may occur in the urine and their clinical significance: indicanuria, melanuria, the diazo reaction, bilirubinuria, urobilinuria.

8. The microscopic study of the urine: technique; the non-organized components of urinary sediments; the significance of such deposits.

9. The microscopic study of the urine: the organized components of urinary sediments—tube casts, pus, blood, epithelium.

10. The bacteriological study of the urine: renal tuberculosis and its diagnosis; the typhoid bacillus, the colon bacillus; technique.

G. **The Milk.**—Milk analysis and its indications.

II. **Laboratory Exercises.** **Blood.**—In all microscopic exercises an examination with the low power (B. and L. $\frac{2}{3}$; Leitz 3; Spencer 16)

half-dozen cells, or but a few more, appear in the field at one time. Make an Arneth count of the neutrophilic elements. Note the myelocytes, metamyelocytes, monokaryolobic leukocytes, polykaryolobic leukocytes; draw. Contrast this type of hyperleukocytosis with that seen in pyogenic infections. Study the staining qualities of the eosinophilic myelocytes and contrast with what is seen in the adult forms; draw. Note the increase of the mast-cells; draw. Look for evidences of nuclear division in the myelocytes. Study the variations in the size of the different leukocytes. Note the presence of isolated large lymphocytes.

Study the nucleated red cells; look for mitoses, nuclei undergoing karyolysis, free nuclei; note the polychromatophilic protoplasm of some of the nucleated red cells.

Exercise X. Study the blood from cases of acute and chronic lymphocytic leukemia (p. 112).

Exercise XI. Study the red cells in stained specimens of blood from a case of chlorosis, a severe secondary anemia the result of a pyogenic infection, chronic lead intoxication, carcinomatous cachexia, pernicious anemia. Compare carefully the size of the cells, their form and their apparent amount of hemoglobin; draw. Look for polychromatophilic red cells, granular degeneration, and nucleated red cells. Contrast megaloblasts and normoblasts in the blood of pernicious anemia and myelocytic leukemia; note especially the size, form, and structure of the nucleus; note small and large (young and old) megaloblasts and young and old normoblasts; nuclei in karyorhexis; draw (pp. 51 to 53, p. 60, and pp. 61 to 69).

Exercise XII. Make a red and white count in a normal individual, using the hemocytometer (pp. 137 to 141). Make a dry normal mount, stain with eosinate of methylene blue, wash with water; examine the specimen while still wet, with a low power (B. and L. ⅔; Leitz 3; Spencer 16), and note the number of blue specks (leukocytes, in the thinner and thicker parts of the spread; note that this is a normal case. Look at mounts from different infections (smeared with a little oil on the surface to obtain satisfactory refraction), and gauge the number of leukocytes by comparing with the normal. Practise this in concrete cases, controlling your findings with the hemocytometer. Make a differential count with the low power and practise this thoroughly.

Exercise XIII. Estimate the amount of hemoglobin with different hemoglobinometers (Fleischl, Dare, Sahli, Talquist) (pp. 147 to 154).

Exercise XIV. Determine the color index in a given case (p. 53).

Exercise XV. Prepare eosinate of methylene blue (p. 129) and some modification of the Romanowsky stain (p. 132).

Exercise XVI. Stain specimens with Hastings', Goldhorn's or Giemsa's stain, and also with Ehrlich's stain (the latter, after fixing by heat) (pp. 130, 132, 136, and 137).

Exercise XVII. Study specimens of malarial blood, stained with a Romanowsky mixture, and also with eosinate of methylene blue. Work out the different stages of development of the malarial organism in the different types of fever. Study, if possible the fresh, unstained blood also (pp. 177 to 187).

Exercise XVIII. Repeat lesson XVII, and examine also dehemoglobinized specimens, prepared according to Ruge's modification of Ross' method (p. 177).

Exercise XIX. Study the distinguishing characterstics of Anopheles, Culex, and Stegomyia mosquitoes; examine their eggs, larvæ, and pubæ.

Exercise XX. Study preparations showing the development of the malarial organism in the body of the mosquito.

Exercise XXI. Study preparations of trypanosomes, Leishmania-Donovani, recurrens spirochætæ, and filariæ (pp. 187, 169, 190, and 191).

Exercise XXII. Make the agglutination test in a well-advanced case of typhoid fever (pp. 161 and 164).

Exercise XXIII. Make a bacteriological examination of the blood in a case of typhoid fever (pp. 158 and 159).

Exercise XXIV. Determine the opsonic index in a given case (*a*) for the Staphylococcus aureus, (*b*) for the tubercle bacillus (p. 646).

B. **Gastric Contents.**—Prepare an artificial "gastric juice" according to the following formula: 0.3 per cent. solution of hydrochloric acid, 500 c.c.; pepsin powder, 2 grms.; bread, 40 grms.

Place this in the incubator for thirty minutes, filter, and then examine as follows:

Exercise I. Test the reaction with litmus paper; test for free acid with Congo red (p. 219); test for free HCl with dimethyl-amino-azobenzol (p. 219), phloroglucin vanillin (p. 220), and tropæolin (p. 221).

Determine the acid factors:

(*a*) The total acidity in 10 c.c., and express your results in terms of c.c. of $\frac{n}{10}$ alkali solution for 100 c.c. of gastric juice (p. 222).

(*b*) The alizarin acidity in a similar portion (free acids and acid salts); deduct this value from the total acidity; the result gives the acidity referable to combined HCl (p. 222).

(*c*) The free HCl in a third portion of 10 c.c. (p. 222); *b* plus *c* gives the total amount of HCl; this deducted from *a* indicates the acidity due to acid salts.

Apply the biuret test to 10 c.c. of stomach contents: render strongly alkaline with caustic alkali, and add a 2 per cent. solution of $CuSO_4$ solution, drop by drop.

Exercise II. Repeat exercise I and demonstrate further the presence of pepsin (p. 229) and of rennin (p. 231). Estimate the amount of pepsin according to Metts' method (p. 229).

Exercise III. Prepare an acid mixture as above, substituting lactic acid for the hydrochloric acid. Apply (*a*) Kelling's test, (*b*) Uffelmann's test (after extracting with ether); estimate the amount of lactic acid according to Boas' briefer method (pp. 235 and 240).

Exercise IV. Secure stomach contents from a case of carcinoma of the stomach, after Boas' test meal. Demonstrate the presence of lactic acid and estimate its amount; show the absence of free HCl; test for pepsin and rennin, viz., pepsinogen and renninogen.

Examine the sediment of the contents, by mounting a drop on a slide and covering with a cover glass; note the starch granules (stain with Lugol's solution); search for Boas-Oppler bacilli in a whet specimen and in a smear stained with a 1 per cent. aqueous solution of methylene blue; so also for yeast cells and bacteria in general; look for pus corpuscles (neutrophilic leukocytes), red cells, and protozoa (p. 249).

Exercise V. Examine the contents from exercise IV for occult blood (p. 260).

Exercise VI. Procure stomach contents from a case of dilatation in the morning before food has been taken. Note the amount of fluid and of residual food material. Examine microscopically and chemically as above. If much HCl is present sarcinæ may be found; if not, examine a droplet of an emulsion of sarcinæ obtained from a culture; also make a smear and stain with methylene blue.

Exercise VII. Prepare a $\frac{n}{10}$ solution of sodium hydrate and standardize it against $\frac{n}{10}$ oxalic acid (p. 216).

C. Feces.—*Exercise I.* Procure some normal feces, stir up with normal salt solution to a thin mush; mount droplets, further diluted, on slides, and cover with cover-glasses. Note that the feces are largely composed of bacteria. Here and there muscle fibers may be found, more or less well preserved and all stained yellow. Look for fat globules and stain with a drop of Sudan III solution (fat-red) (p. 266); search also for starch (add a drop of Lugol's solution; starch colored blue); look for fatty acid needles; draw.

Make smears from the same stool and stain with methylene blue; note the great variety of bacteria; draw.

Exercise II. Repeat exercise I with a diarrheal stool. Look for particles of mucus and pus. Examine wet specimens and smears, stained with methylene blue; draw.

Exercise III. Provide stools containing ova of the hookworm, tapeworm, and Trichocephalus dispar; study and draw these; furnish eggs from other intestinal parasites; draw (pp. 295 to 316).

Study the corresponding worms; draw.

Exercise IV. Provide trichinous meat and study the corresponding parasite (p. 311); review exercise III.

Exercise V. Study the Amœba coli in suitable stools (vital staining with neutral red); so also the trichomonas; draw. Such stools cannot be adequately preserved, while most others can be kept in 1 per cent. carbolic acid (pp. 286 and 291).

Exercise VI. Provide a stool containing blood and pus; note especially the size of the pus cells; examine microscopically; demonstrate the presence of blood chemically (p. 260).

D. **Sputum.**—In working with sputa treat all specimens as though they were tuberculous.

Exercise I. Examine wet mounts of scrapings from the tongue and teeth; note the character of the epithelium, mucous corpuscles, and the large number and variety of microörganisms (among these spirochetæ). Prepare smears on slides and stain with methylene blue; draw.

Exercise II. Examine sputa from cases of acute and chronic bronchitis, asthma, and chronic heart lesions as in I; note the epithelial elements (myelin, fat, and pigment granules), pus cells, red corpuscles, crystals. In stained specimens search for eosinophiles and note their number (pp. 334 to 337).

Exercise III. Examine tuberculous sputa for the tubercle bacillus, according to Gabbett's method; examine for elastic tissue (procure material from an advanced case with cavity formation) (pp. 337 and 345).

Exercise IV. Examine pneumonic sputa; note their physical characteristics, color, and odor; demonstrate the pneumococcus and attempt its isolation. Stain the capsules according to Bürger's method (p. 348).

E. **Exudates.**—*Exercise I.* Prepare smears from tonsillar deposits and stain with alkaline methylene-blue solution; note the presence of staphylococci, streptococci, and diphtheria bacilli; examine cultures of the latter grown on blood serum; make smears and stain according to Neisser's method; note the polar bodies; draw (p. 205).

Study specimens from Vincent's angina (spirilla and fusiform bacilli) (p. 204).

Exercise II. Prepare smears of pus from an acute case of gonorrhea; stain a specimen with eosinate of methylene blue; note (*a*) the gonococci in the pus cells (neutrophilic leukocytes); (*b*) the presence of eosinophilic leukocytes. Stain another specimen according to Gram. (p. 602).

Obtain some gonorrheal threads from the urine; make smears of these and examine in the same manner; draw.

Exercise III. Obtain smears of syphilitic serum from chancres, papules, and condylomata and stain according to Goldhorn's method; note the S. refringens and the pallida; contrast the two and draw (p. 606).

Exercise IV. Procure a serous pleural exudate, determine its cytological formula, and search for organisms (tubercle bacilli) according to Jousset's method. Also determine the specific gravity of the fluid and the amount of albumin (pp. 592 and 596).

Contrast these various factors with a transudate.

Exercise V. Examine cerebrospinal fluid in the same manner as in IV.

Exercise VI. Examine by culture methods a specimen of an exudate obtained at operation (peritonitis).

F. **The Urine.**—*Exercise I*. Collect the urine of twenty-four hours from a normal individual, preserve with a teaspoonful of chloroform, which should be added to the first portion voided, shaking well after every addition. Note the color, odor, and transparency, its reaction to litmus, and specific gravity. Estimate the acidity according to Folin's method (pp. 360 to 373).

Estimate the chlorides according to Volhard's method (p. 377).

Exercise II. Estimate the urea (p. 411) and uric acid (p. 424).

Exercise III. Examine microscopically the sediment of a dozen specimens of normal urine (p. 546); note the epithelial elements (especially in the urine of women), their number and form, crystals and mucous cylinders; draw. Let one specimen stand exposed to the air without a preservative for forty-eight hours and reëxamine; note the development of bacteria and the abundant sediment; add acetic acid and note that the amorphous portion largely dissolves. How would you distinguish such a sediment from one due to amorphous urates?

Exercise IV. Procure urine from a case of active nephritis. Examine for albumin, using the various tests described on pp. 461 to 466. Estimate the amount in an Esbach tube (p. 467). Examine microscopically for tube casts (p. 570).

Exercise V. Procure urine from a patient after a somewhat prolonged ether anesthesia and examine as in IV.

Exercise VI. Procure urine from different cases of nephritis and examine as in IV; contrast the findings in an arteriosclerotic case with those in an acute process, in association with typhoid fever or scarlet fever. Stain some of the sediment on the slide with a little eosin and note how the different kinds of casts are colored. Draw hyaline, granular, brown, epithelial, pus, blood, and waxy casts.

Exercise VII. Add 10 grams of glucose to 500 c.c. of urine and examine qualitatively for sugar, using (*a*) Fehling's test, (*b*) Nylander's test, (*c*) the fermentation test, (*d*) the phenylhydrazin test (purify the osazone crystals in the latter case and determine their melting point (pp. 483 to 487). Estimate the quantity of sugar (*a*) by Fehling's method (p. 489), (*b*) with the polarimeter (p. 494).

Exercise VIII. Repeat exercise VII with the urine from an active case of diabetes; test also for acetone directly; distil some of the urine and demonstrate acetone in the distillate (Lieben's test, Legal's test, Gunning's test, etc., p. 529). Test for diacetic acid. Add some β-oxybutyric acid to the urine, demonstrate its presence, and estimate the amount (p. 532).

Exercise IX. Procure urine from a typhoid patient, about the tenth day of the disease. Apply the diazo test (p. 524) and also the indican test (p. 503). Test normal urine in the same way and compare the colors. In doing the diazo reaction do not fail to practise the diluting method in the end and compare the color with the normal.

Exercise X. Procure urine from a case of jaundice and apply the various tests for bile pigment (p. 512); contrast the results with a urine rich in urobilin; demonstrate the presence of the latter (p. 515).

Exercise XI. Procure urine from cases of cystitis and pyelitis and study these microscopically (pus corpuscles, red cells, epithelial cells). Examine for tubercle bacilli by Gabbett's method and inject a guinea-pig with sediment from a tuberculous case (autopsy after three weeks).

Exercise XII. Show some of the rarer crystalline elements, such as cystin, leucin, tyrosin, fatty acids, xanthin, hippuric acid, etc., and review sediments in general thoroughly.

G. **Milk.**—*Exercise I.* Determine the specific gravity of a specimen of milk. Estimate the amount of fat (p. 637), of lactose (p. 637), and of proteids (p. 637).

Examine a droplet of milk microscopically; note the fat globules and their behavior toward Sudan III.

Exercise II. Examine colostrum as in exercise I.

INDEX.

A

ABORTION, vaginal discharge in, 631
Acetic acid, 241
  fermentation, 240
  tests for, 241
Acetone in the blood, 50
  in the gastric contents, 244
  in the urine, 527
  quantitative estimation of, 531
  tests for, 529
Acetonemia, 50
Acetonuria, 527
Acholic stools, 258, 262, 267
Acids, fatty, in the feces, 319
  organic, in the gastric contents, 240
Actinomyces hominis, 202, 351
Actinomycosis, 351
Adenin in the urine, 427, 585
Agar. *See* Culture media.
Agglutination test, 160
Agglutinins, 160
Albumin, acetosoluble, 454
  in the feces, 269, 323
  in the urine, 440
  quantitative estimation of, 467
  residual, in feces, 269
  special test for serum albumin, 466
    for serum globulin, 469
  tests for, 461
    boiling, 464
    nitric acid, 462
    picric acid, 466
    potassium ferrocyanide, 465
    Spiegler's, 466
    trichloracetic acid, 465
Albuminimeter, 467
Albuminous expectoration, 358
Albuminuria, 440
  accidental, 453
  colliquative, 448
  constitutional, 444
  cyclic, 443
  digestive, 452
  febrile, 446
  functional, 442
  hematogenous, 444, 450
  in organic diseases of the kidneys, 445
  intermittent, 442
Albuminuria, mixed, 453
  neurotic, 451
  orthostatic, 443
  physiological, 441
  postural, 443
  referable to circulatory disturbances, 449
    to impeded outflow of urine, 450
  renal, 445
  toxic, 451
  transitory, 442
Albumoses in the blood, 40
  in the feces, 323
  in the gastric contents, 232
  in the urine, 454
  tests for, 469
Albumosuria, 454
  digestive, 455
  enterogenic, 454
  hematogenic, 455
  hepatogenic, 454
  histogenic, 455
  mixed, 455
  pyogenic, 454
  renal, 455
  vesical, 455
Alimentary detritus in feces, 261, 266
Alkalimeter, Engel's, 21
Alkaline stools, 316
  urine, 369
Alkalinity of the blood, 20
  estimation of, 21
Alkapton in the urine, 518
Alkaptonuria, 518
Alloxur bases in the urine, 418, 427
  estimation of, 427
Almén's solution, 484
Aloin test for occult blood, 260
Alveolar epithelium, 336
Amino-acids in the urine, 538
Ammonia in the blood, 43
  in the gastric contents, 242
  in the urine, 415
    estimation of, 416
Ammoniacal fermentation, 369
Ammoniomagnesium phosphate, 560
Ammonium urate, 560
Amebas in the urine, 586
Amœba coli, 286

Amœba coli in the feces, 286
  in the sputum, 339
Amœbina in the feces, 286
Amphistomum hominis, 304
Amphoteric urine, 369
Amyloid corpuscles in the semen, 624
Anachlorhydria, 218
Anacidity, hysterical, 218
Anadeny of the stomach, 218, 244
Anemic degeneration of the red corpuscles, 60
Anguillula aceti, 586
  intestinalis, 312
  stercoralis, 312
Anguilluliasis, 196
Aniline dyes, classification of, 125
  -water, gentian violet, 346
Animal parasites in the blood, 177
  in the feces, 285
  in the sputum, 339
  in the urine, 586
Anisocytosis, 52
Anisohypercytosis, 101
Anisohypocytosis, 101
Anisonormocytosis, 101
Ankylostomiasis, 307
Ankylostomum duodenale, 307
Annelides, 305
Anthracosis of the lungs, 357
Anthrax, bacillus of, 172
Apiosoma of typhus fever, 190
Arabinose in urine, 496
Arneth's karyomorphism, 76, 81
Arnold's test for diacetic acid, 533
Ascarides in the feces, 305
  in the urine, 586
Ascaris lumbricoides, 305
  maritima, 305
  mystax, 305
  Texana, 307
Asiatic cholera, bacillus of, 283
Aspergillus fumigatus, 353
Asthma, bronchial, Charcot-Leyden crystals in, 333
Azoöspermatism, 625
Azurophilic granulation, 72

B

BACILLUS acidophilus, 280
  coli communis, 282
  dysenteriæ, 276
  lactis aërogenes, 283
  of anthrax, 172
  of cholera Asiatica, 283
  of diphtheria, 205
  of dysentery, 276
  of Finkler and Prior, 285
  of glanders, 174
  of influenza, 349
  of leprosy, 345
  of Moro, 280
  of Oppler and Boas, 250
  of paratyphoid fever, in the blood, 165
    in the feces, 280
  of plague, 175, 351
  of Shiga, 276
  of smegma, 351
  of tuberculosis, in the blood, 173
    in the feces, 285
    in the gastric contents, 249
    in the meningeal fluid, 616
    in the milk, 634
    in the mouth, 202
    in the nasal discharge, 325
    in the sputum, 344
    in the urine, 580
    methods of staining, 345
  of typhoid fever, in the blood, 15
    in the feces, 278
    in the sputum, 351
    in the urine, 584
  of whooping-cough, 350
  pestis, 175
  pyocyaneus, 282
  smegma, 351, 583
  vulgaris, 281
Bacteria in blood, 158
  in exudates, 595
  in feces, 273
  in gastric contents, 250
  in milk, 634
  in mouth, 202
  in nasal secretion, 325
  in pus, 600
  in saliva, 200
  in sputum, 344
  in urine, 580
  in vagina, 627
Bacterial decomposition of the urir 369
Bacteriemia, 158
  colon, 171
  gonococcus, 171
  meningococcus, 171
  paracolon, 165, 171
  paratyphoid, 165
  pneumonia, 166, 170
  proteus, 170
  pyocyaneus, 171
  pyogenic, 168
  staphylococcus, 168
  streptococcus, 169
  typhoid, 159
Bacteriuria, 580
  idiopathic, 585
Bacterium lactis aërogenes, 283
  proteus, 170
Balantidium coli, 293
Bang's test for albumoses, 470
    for urobilin, 470
Barfoed's reagent, 233
Barfurth's reagent, 137
Basic phosphate of magnesium, 558,

Basophilic leukocytes in the blood, 78
    in the sputum, 334
  perinuclear granules, 76
Baumann and v. Udranszky's method of isolating diamins, 543
Baumann's test for homogentisinic acid, 521
Beckmann's apparatus, 156
Bence Jones' albumin, 456
    tests for, 471
Benzoic acid in the urine, 431
Bile pigment in the blood, 49
  in the feces, 321
  in the gastric contents, 246
  in the urine, 510
    tests for, 512
      Gmelin's, 512
      Huppert's, 512
      Rosenbach's, 512
      Smith's, 512
Bilharzia hematobia, 195
    eggs in the urine, 586
Bilharziasis, 195
Biliary acids in the blood, 50
    in the feces, 320
    in the urine, 512
    tests for, 320
  concretions, 263
Bilirubin, 510
Biuret test, 407
Blastomycetes, 353
Blood, 17
  acetone in, 50
  albumins in, 39
  albumoses in, 40
  alkalinity of, 21
  ammonia in, 43
  bacteriology of, 158
  biliary constituents in, 49
  carbohydrates in, 40
  chemical examination of, 25
  coagulation of, 27
  color of, 17
  color index, 53
  corpuscles, red, 51
    anemic degeneration of, 60
    behavior toward aniline dyes, 60
    color index, 53
    crenation of, 53
    granular degeneration of, 61
    enumeration of, 137
    money-roll formation, 53
    nucleated, 65
    osmotic resistance of, 157
    polychromatophilia of, 60
    ring bodies in, 64
    variations in color, 53
      in form, 52
      in number, 54
      in size, 51
  counting, 137
  crisis, 66
Blood, drying and staining of, 123, 125
  dust, 120
  examination, technique of, 119
  fat in, 45
  fatty acids in, 45
  fibrin in, 39
  gases in, 26
  general characteristics of, 17
    chemistry of, 25
  glycogen in, 42
  hemokonia of, 120
  in the feces, 259, 271
  in the gastric contents, 247
  in the sputum, 328, 335
  in the urine, 508, 567
  iron, 153
  kryoscopy of, 154
  lactic acid in, 48
  leukocytes of, 69
  medicolegal test for, 36
  methods of staining, 125
  microscopic examination of, 51
  mount, 121
  nucleated corpuscles in, 65
  occult, in feces, 260
  odor of, 18
  parasitology of, 177
  peptone in, 40
  pigments of, 29
  plaques of, 118
  plates, 118
  proteids in, 39
  protozoa in, 177
  reaction of, 20
  red corpuscles, 51
  shadows, 568
  specific gravity of, 18
  staining of, 125
  sugar in, 40
  tests for, 36, 260
    aloin test, 260
    Donogany's, 247, 474
    guaiacum, 261
    Heller's, 473
    Müller and Weber's, 247
  uric acid in, 43
  volume index, 147
  white. *See* Leukocytes.
  xanthin bases in, 44
Boas' bulbed stomach tube, 211
  method for estimating lactic acid, 238
  test for hydrochloric acid, 221
    for lactic acid, 237, 240
    meal, 210
Boas-Oppler bacillus, 250
Bogg's method of estimating proteids in milk, 637
Boiling test for albumin, 464
Bothriocephalus latus, 300
Böttcher's crystals, 623
Böttger's test for sugar, 484
Bremer's diabetic blood test, 61

Brick-dust sediments, 549
Brodie and Russell's method of enumerating the plaques, 144
Browning's spectroscope, 23
Bubonic plague, bacillus of, 175
Buccal secretion (*see* Saliva), 198
Buerger's capsule stain, 348
Butyric acid fermentation, 241
    in the feces, 319
    in the gastric contents, 241
        test for, 241

C

Cabot's ring bodies, 64
Cadaverin, 543
Cahn-Mehring's method of estimating fatty acids, 242
Calcium carbonate, crystals of, 560
    oxalate, crystals of, 357, 551
    phosphate, crystals of, 552
    sulphate, crystals of, 553
Carbohydrates, digestion of, 233
    in the blood, 40
    in the feces, 323
    in the urine, 474
    tests for, 233
Carbol fuchsin, 345
Carbolic acid, estimation of, 517
    test for, 318, 517
Carbolochloride of iron test for lactic acid, 235
Carbon dioxide hemoglobin, 35
    monoxide hemoglobin, 34
Casein, in the milk, 637
    test for, Leiner's, 269
Casts, blood, 572
    classification of, 571
    epithelial, 572
    examination of, 571
    fatty, 574
    fibrinous, 330
    formation of, 570
    granular, 574
    hyaline, 572
    pus, 565
    significance of, 577
    staining of, 572
    urinary, 570
    waxy, 574
Cellulose in the blood, 42
Cenomonadina, 289
Cercomonas intestinalis, 290
Cerebrospinal fluid, 609
    amount of, 610
    appearance of, 610
    bacteriology of, 616
    chemical composition of, 612
    cholin in, 613
    cytodiagnosis of, 615
    microscopic examination of, 615
Cerebrospinal fluid, reaction of, 612
    specific gravity of, 610
    toxicity of, 618
Cestodes, 295
Chalicosis, 358
Charcot-Leyden crystals, in the feces, 272
    in the nasal discharge, 325
    in the sputum, 333
Cheesy particles in sputum, 330
Chemical examination of blood, 25
    of cystic fluids, 619
    of exudates, 597
    of feces, 316
    of gastric juice, 214
    of milk, 636
    of pus, 598
    of saliva, 198
    of semen, 624
    of sputum, 358
    of transudates, 590
    of urine, 373
Chlorides in the urine, 374
    estimation of, 377
        according to Salkowski and Volhard, 377
        direct method, 381
    test for, 377
Cholemia, 49
Cholera Asiatica, bacillus of, 283
    infantum, bacillus of, 276
Cholesterin in the blood, 47
    in the feces, 272, 319
    in the sputum, 356
    in the urine, 513
    isolation of, from the feces, 319
    test for, 319
Cholin in the blood, 50
Choluria, 510
Chorion villi, 631
Chromogens in the urine, 501
Chyluria, 539
Chymosin, 230
    estimation of, 232
    test for, 231
Chymosinogen, 230
    estimation of, 232
    test for, 231
Ciliata, 293
Cladothrix asteroidea, 352
Coagulation of the blood, 27
Coagulometer, Wright's, 28
Coating of the tongue, 203
Coffin-lid crystals, 560
Colloid concretions in ovarian cysts, 620
Color index of the blood, 53
Colostrum, 632
Comma bacillus, 283
Concretions, biliary, 263
    fecal, 264
    intestinal, 263
    pulmonary, 333

Congo-red test for free acids, 219
Conjugate glucuronates, 497
  sulphates, 392
Coproliths, 264
Corpora amylacea, 624
Crenation of red corpuscles, 53
Crotonic acid, 535
Crystals, ammoniomagnesium phosphate, 560
  ammonium urate, 560
  bilirubin, 557
  calcium carbonate, 560
    oxalate, 551
    phosphate, 552
    sulphate, 553
  Charcot-Leyden, 354
  cholesterin, 356
  cystin, 553
  fatty acids, 365
  hematoidin, 557
  hemin, 36
  hippuric acid, 552
  indigo, 561
  in the feces, 271
  leucin, 554
  leukocytic, 354
  magnesium phosphate, 558, 559
  monocalcium phosphate, 552
  neutral calcium phosphate, 558, 559
  phenylglucosazone, 486
  phosphate of spermin, 621
  Teichmann, 36
  triple phosphate, 560
  tyrosin, 554
  urate of ammonium, 560
  uric acid, 549
  xanthin, 556
Culture media (preparation of), 649
Curschmann's spirals, 332
Cylinders, mucous, in the feces, 262
    in the urine, 577
  urinary, 571
Cylindroids, 576
Cylindruria, 571
Cystein, 396
Cysticercus cellulosæ, 297
Cystin, 397, 553
Cystinuria, 397, 553
Cysts, colloid, 620
  contents of, 619
  dermoid, 620
  fibrocystic, 621
  hydatid, 621
  ovarian, 619, 621
  pancreatic, 622
  parovarian, 621
Cytodiagnosis in cerebrospinal fluid, 615
  in effusions, 592
Czaplewsky's carbol fuchsin, 345

D

DALAND'S hematokrit, 144
Dare's hemo-alkalimeter, 22
    hemoglobinometer, 148
    method of estimating the alkalinity of the blood, 22
Decidual cells, 631
Decinormal alkali, preparation of, 216
Dehemoglobinizing method, 177
Dennigè's test for acetone, 530
Dermoid cysts, 620
Dextrin in the urine, 496
Dextrose in the urine. *See* Glucose.
Diabetes, 481
  alternans, 423
  Bremer's blood test in, 61
  hepatogenic, 482
  Hirschfeld's form of, 483
  insipidus, 377, 498
  myogenic, 482
  phosphatic, 384
  Williamson's blood test in, 42
Diabetic chromatophilia, 61
Diacetic acid in the urine, 532
    tests for, 532
Diaceturia, 532
Diamins in the feces, 323
  in the urine, 543
    isolation of, 543
Diathesis, oxalic acid, 438
  uric acid, 421
Diazo reaction (*see* Ehrlich's reaction), 522
Dibothriocephalus latus, 300
Differential density method of estimating sugar, 491
  leukocyte count, 143
Digestion, products of, 232
Dimethylaminoazobenzol test, 219
Diphtheria, 205
Diplococcus meningitidis intracellularis, 616
  pneumoniæ, 348
    in the blood, 166
Dipylidium caninum, 299
Distoma Buskii, 304
  capense, 195
  conjunctum, 304
  hematobium, 195, 343
  hepaticum, 302
  heterophyes, 305
  lanceolatum, 303
  pulmonale, 343
  rhatonisi, 304
  sibiricum, 304
  spatulatum, 304
Distomiasis, 195
Donné's pus test, 564
Donogany's blood test, 247, 474
Doremus' ureometer, 411
Drigalsky-Conradi medium, 278
Drysdale's corpuscles, 621

Dunlop's method of ematisting oxalic acid, 439
Dust particles of Müller, 120
Dyes, 125
    neutral, 127
    polychrome, 127
Dysmenorrhea exfoliativa, 630

E

EARTHY phosphates, 382
Eberth's bacillus, 278
Echinococcus, 339
    membranes in the sputum, 333
        in the urine, 587
    polymorphus, 339
Egg-yellow reaction, 525
Ehrlich's diazo reaction, 522
    dimethylaminobenzaldehyde reaction, 526
    egg-yellow reaction, 525
    hemoglobinemic Innenkörper, 65
    triacid stain, 130
Einhorn's saccharimeter, 492
Elastic tissue in the sputum, 330, 337
        stain for, 339
Endocarditis, bacteriology of, 171
Engel's alkalimeter, 21
    method of estimating the alkalinity of the blood, 21
Entamœba coli, 289
    dysenteriæ, 286
    histolytica, 286
        in the feces, 286
        in the sputum, 339
Enterogenic albumosuria, 454
Enteroliths, 264
Eosinate of methylene blue, staining with, 129
Eosinophilia, 102
Eosinophilic leukocytes in the blood, 77
        in the sputum, 334
Epithelial cells, alveolar, 336
        ciliated, 336
        in the buccal secretion, 200
        in the feces, 270
        in the sputum, 336
        in the urine, 561
        in the vaginal secretion, 627
Erythroblasts, 65
Erythrodextrin, 233
    test for, 233
Esbach's albuminimeter, 467
    method of estimating albumin, 467
    reagent, 467
Escherich's stain, 281
Ethyl sulphide, 397
Euchlorhydria, 218
Eustrongylus gigas, 586
Ewald and Siever's salol test, 253.
Ewald's test breakfast, 210
Exudates, 591
Exudates, bacteriological examination of, 595
    chemistry of, 597
    chyloid, 604
    chylous, 604
    cytodiagnosis of, 592
        technique, 594
    hemorrhagic, 591
    in cancer, 594
    in tuberculosis, 592
    inoscopy, 596
    purulent (*see* Pus), 598
    putrid, 604
    serous, 591

F

FARRANT'S solution, 571
Fat in the blood, 45
        estimation of, 46
    in the feces, 262
    in the milk, estimation, 637
    in the urine, 539, 557
Fatty acids, estimation of, 242
        formation of, 240
        in pus, 602
        in the blood, 45
            estimation of, 47
        in the feces, 319
        in the gastric contents, 240
        in the sputum, 356
        in the urine, 537
            estimation of, 537
        tests for, 241
    casts, 574
Febrile acetonuria, 528
    albuminuria, 446
    urobilin, 513
Fecal vomiting, 248
Feces, 256
    albumin in, 323
    albumoses in, 323
    alimentary detritus in, 261
    amount of, 256
    animal parasites in, 285
    annelides in, 305
    bacteriology of, 273
    biliary acids in, 320
        concretions in, 263
        pigments in, 231
    blood in, 259, 271
    cestodes in, 295
    chemistry of, 316
    cholesterin in, 319
    color of, 258
    composition of, 317
    concretions in, 263
    consistence of, 257
    coproliths in, 264
    crystals in, 271
    enteroliths in, 263
    epithelial cells in, 270

Feces, fatty acids in, 319
  flagellata in, 289
  foreign bodies in, 265
  form of, 257
  gases in, 317
  hematoporphyrin in, 322
  indol in, 318
  intestinal concretions in, 264
    sand in, 264
  leukocytes in, 271
  macroscopic constituents of, 261
  microscopic constituents of, 265
  mucus in, 262, 273, 322
  number of stools, 256
  odor of, 258
  parasites, animal, 285
    vegetable, 273
  phenol in, 318
  pigments in, 320
  protozoa in, 286
  ptomains in, 323
  purin bodies in, 322
  reaction of, 316
  residual albumin in, 269
  skatol in, 318
  technique in examination of, 265
  trematodes in, 302
  vermes in, 305
Fehling's method of estimating sugar, 489
  solution, 484
  test for sugar, 484
Ferment, milk-curdling, 230
  of saliva, 199
Fermentation test for sugar, 485
    Schmidt's fecal, 268
Ferments in the gastric juice, 227
  in the urine, 540
Ferrocyanide test for albumin, 465
Ferrometer, Jolles', 153
Fibrin, 39
  estimation of, 39
  ferment, 27
  in the blood, 39
  in the urine, 459
  test for, 474
Fibrinogen, 27
Fibrinous casts, 330
  coagula in the sputum, 330
  in the urine. *See* Chyluria.
Ficker's diagnosticum, 164
Filaria Bancrofti, 191
  demarquai, 191
  diurna, 191
  Mansoni, 191
  nocturna, 191
  ozzardi, 191
  perstans, 191
  sanguinis hominis, 191
  Wuchereri, 191
Filariasis, 191
Finkler-Prior bacillus, 285
Fixation, 124
Flagellata, 289
Fleischl's hemometer, 150
Florence's test for semen, 626
Folin's method of estimating the acidity of the urine, 371
    ammonia, 416
    kreatinin, 434
    sulphates, 394, 395
    urea, 412
    uric acid, 424
Foreign bodies in the feces, 265
  in the sputum, 333
  in the urine, 587
Frommer's test, 530
Furfurol test for bile acids, 320
Fusiform bacilli of Vincent, 204

G

Gabett's staining method, 345
Galacturia, 539
Gallstones in the feces, 263
Garrod's test for hematoporphyrin in the urine, 509
    for homogentisinic acid, 520
Gases in the blood, 26
  in the feces, 317
  in the gastric contents, 242
  in the urine, 541
Gastric contents, examination of (*see* Gastric juice), 209
    products of digestion, 232
      analysis of, 232
  juice, 209
    acetic acid in, 241
    acetone in, 244
    acidity of, 214
    amount of, 213
    blood in, 247
    butyric acid in, 241
    cause of acidity of, 214
    chemical composition of, 214
      examination of, 214
    chymosin in, 230
    chymosinogen in, 230
    combined hydrochloric acid in, 222
    fatty acids in, 240
    ferments in, 227
    free acid in, 217, 219
    gases in, 242
    general characteristics of, 213
    hydrochloric acid in, 217
    hyperacidity of, 218
    hypersecretion of, 218
    lactic acid in, 233
    methods of obtaining, 211
    microscopic examination of, 249
    milk-curdling ferment of, 230
    organic acids in, 240
    pepsin in, 227

Gastric juice, pepsinogen in, 227
  rennin, 230
  secretion of, 209
  zymogens in, 227
Gastrosuccorrhea mucosa, 246
Gelatin (*see* Culture media), 649
Gerhardt's test for diacetic acid, 532
  for urobilin, 515
Gerrard and Allan's estimation of sugar, 490
Giemsa's stain, 136
Gigantoblasts (*see* Megaloblasts), 67
Glanders, bacillus of, 174
Glandular fever, 208
Glaser's method of estimating neutral sulphur, 398
Glucose, 474
  in the blood, 40
    estimation of, 40
  in the urine, 474
  quantitative estimation of, 489
  tests for, 483
Glucosuria, 474
  digestive, 474
  e saccharo, 474
  ex amylo, 478
  persistent, 481
  transitory, 479
Glucosuric acid. *See* Alkapton.
Glucuronic acid in the blood, 40
    in the urine, 497
Glycogen in the blood, 42
      test for, 137
Gmelin's reaction, 512
Goldhorn's stain, 137
Gonococcus in the blood, 171
  in the mouth, 203
  in the urine, 585
  in urethral discharge, 603
  Neisser's, 603
    staining of, 585
Gonorrheal pus, 602, 630
  stomatitis, 202
  threads in the urine, 566
Gowers' hemoglobinometer, 152
Gram's method of staining, 205
Granular degeneration, 61
  casts, 574
Granulocytes, 69
Grape sugar. *See* Glucose.
Gregarina, 294
Guaiacum test for blood, 261
Guanin in the urine, 427
Gunning's mixture, 413
    test, 530
Günzburg's packages, 254
  reagent, 220

H

HALITUS sanguinis, 18
Hammerschlag's method of determining the specific gravity of blood, 18
Hammerschlag's method of estimating pepsin, 229
Hastings' stain, 132
Heart-disease cells, 337
Heller's test for albumin, 462
    for blood, 473
Hematemesis, 248
Hematin, 35
Hematinuria, 458, 508
Hematoblasts, 118
Hematoidin in the blood, 38
  in the sputum, 356
  in the urine, 557
Hematokrit, 144
Hematoporphyrin in the blood, 38
  in the feces, 322
  in the urine, 508
  tests for, 509
Hematoporphyrinuria, 508
Hematuria, 508, 567
Hemin (*see* Teichmann's crystals), 36
Hemo-alkalimeter, Dare's, 22
Hemochromogen, 29
Hemocytometer of Thoma-Simon, 137
Hemoglobin, 29
  carbon dioxide, 34
    monoxide, 34
  estimation of, with Dare's instrument, 148
    with Fleischl's hemometer, 150
    with Gowers' hemoglobinometer, 152
    with Miescher's instrument, 152
    with Sahli's instrument, 152
    with Talquist's method, 152
  nitric oxide, 35
    sulphohemoglobin, 35
  tests for, 38, 458
Hemoglobinemia, 38
Hemoglobinometer, 147
Hemoglobinuria, 458
    tests for, 473
Hemokonia, 120
Hemometers, 147
Hemospermia, 625
Hepatogenic icterus, 511
Heteroxanthin in the urine, 427
Hippuric acid in the urine, 429,
      estimation of, 431
    properties of, 430
    test for, 431
Histon in the urine, 461
  test for, 474
Hoffmann's test for tyrosin, 556
Hofmeister's method of estimating hippuric acid, 432
  test for leucin, 556
Homogentisinic acid in the blood, 49
    in the urine, 518
      estimation of, 520
      isolation of, 520
Hopkins' method of estimating uric acid. *See* Folin's method.

Huppert's test for bile pigment, 512
Hydatid cysts, 339, 621
      echinococcus membranes and hooklets in, 339, 621
      sodium chloride in, 621
      succinic acid in, 621
  disease, 339
Hydrobilirubin, 320
Hydrocele agar. *See* Culture media.
  fluid, 50
      cholesterin in, 590
Hydrochloric acid in the gastric juice, 214
      amount of, 217
      combined, 222
      deficit, estimation of, 223
      estimation of, according to Leo, 226
        according to Martius and Lüttke, 224
        according to Sahli, 223
        according to Töpfer, 222
      free, 219
      tests for, 219
Hydrogen sulphide, in the gastric contents, 242
          tests for, 243
      in the urine, 541
Hydronephrosis, 622
Hydrothionuria, 541
Hypalbuminosis, 39
Hypeosinophilia, 108
Hyperalbuminosis, 39
Hyperchlorhydria, 218
Hyperchromemia, 31
Hyperinosis, 39
Hyperleukocytosis, 85
  polynuclear eosinophilic, 102
      neutrophilic, 85
Hypersecretio acida et continua, 216, 218
Hypersecretion, 214, 218
Hypinosis, 39
Hypobromite method of estimating urea, 409
  solution, 409
Hypochlorhydria, 218
Hypoleukocytosis, 85
  polynuclear eosinophilic, 108
      neutrophilic, 97
Hypoxanthin in the urine, 427

I

Icterus, 511
  hematogenic, 511
  hepatogenic, 511
  neonatorum, 511
  urobilin, 514
Idiopathic bacteriuria, 585
Ilasvay's reagent, 199
Indican in the urine, 501
Indican in the urine, estimation of, 504
      tests for, 503
Indicanuria, 501
Indigo-blue in the urine, 521, 561
Indigosuria, 521, 561
Indol in the feces, 318
      tests for, 319
Indoxyl, 501
  sulphate (*see* Indican), 501
Influenza, bacillus of, 174, 349
Inoscopy, 596
Inosit in the urine, 498
Intermittent albuminuria, 442
Intestinal concretions, 263
  sand, 264
Iodoform test for lactic acid, 237
Iodophilia, 83
  demonstration of, 137
Iodospermin, 626
Iron in blood, 153
Irritation forms, 82
Isohypercytosis, 101
Isohypocytosis, 101
Isomastigoda, 289
Isonormocytosis, 101

J

Jaffe's test for indican, 504
Jaundice (*see* Icterus), 511
Jenner's stain, 129
Jolles' ferrometer, 153
Justus' syphilitic blood test, 32

K

Kala-azar, Leishmania-Donovani in, 190
Karyomorphism, neutrophilic, 101
Kelling's test for lactic acid, 235
Kernschatten, 75
Kjeldahl's method, 413
Koplik's bacillus, 350
Krabbea grandis, 302
Kreatin, 432
  properties of, 433
Kreatinin, 432
  estimation of, 434
  properties of, 433
  test for, 434
  -zinc chloride, 434
Kryoscopy of the blood, 154
  of the urine, 546

L

Lactic acid 233
      clinical significance of, 233
      estimation of, 238
      fermentation, 235
    in the blood, 48

Lactic acid, in the gastric contents, 233
    in the urine, 535
    mode of formation, 233
    tests for, 235
        Boas', 237
        Kelling's, 235
        Strauss', 235
        Uffelmann's, 235
        Vournaso's, 236
Lactodensimeter of Quevenne, 635
Lactose in the urine, 495
    in the milk, 637
Laiose in the urine, 495
Large mononuclear leukocytes, 73
        clinical variations of, 112
Laveran's organism. ***See* Malarial organism.**
Legal's test for acetone, 529
Leiner's test for casein, 269
Leishmania-Donovani, 190
Leishman's stain, 134
Leo's method of estimating hydrochloric acid, 226
Leprosy, bacillus of, 174
Leptothrix buccalis, 204
Leube's test of motor power of stomach, 252
Leucin, 554
Leucopenia, 97
Leukocytes, 69
    basophilic, 78
    degenerative changes, 75
    differential enumeration, 143
    differentiation according to their behavior toward aniline dyes, 70
    enumeration of, 140
    eosinophilic, 77
    estimation of the number of, 137
    general differentiation of the various forms, 69
    in the blood 69
    in the exudates, 592
    in the feces, 271
    in the sputum, 334
    in the urine, 563
    irritation forms, 82
    large mononuclear, 73
    lymphocytes, 70
    mast-cells, 78
    myelocytes, 80
    neutrophilic, 74
    oxyphilic, 77
    pigmented, 185
    phlogocytes, 82
    polymorphonuclear, 74
    polynuclear, 74
    small mononuclear, 70
    splenocytes, 73
    transition forms, 74
    variations in number of, 84
Leukocytic crystals, 354
Leukocytosis (*see* Hyperleukocytosis), 84
Leukemia, lymphatic, 112
    myelogenous, 116
Levulose in the urine, 495
Lieben's test for acetone, 529
Lientery, 261
Lipacidemia, 46
Lipaciduria, 537
Lipase, in the gastric juice, 232
    in the urine, 540
    test for, 540
Lipemia, 46
Lipuria, 539, 558
Lochia, 629
    alba, 629
    rubra, 629
Löffler's bacillus, 205
    methylene-blue solution, 205
Lohnstein's saccharimeter, 492
Löwy's method of estimating the alkalinity of the blood, 21
Lymphocytes, 70
    small, 70
    large, 71
Lymphocytosis, 73, 109
Lymphopenia, 73, 112

M

Macrocytes, 51
Macrocythemia, 51
Macrolymphocytes, 71
Magnesia, soaps of, in the urine, 557
Magnesium phosphate, 558, 559
Malachite green medium of Lentz, 278
Malaria, plasmodium of, 177
Malta fever, bacillus of, 174
Maltose in the urine, 496
Mammary secretion, 633
Marsh gas in the gastric contents, 242
Martius and Lüttke's method of estimating hydrochloric acid, 224
Masons' lung (*see* Siderosis), 358
Mast-cells, 78
        clinical variations of number, 113
May-Grünwald stain, 130
Meconium, 323
Medicolegal test for blood, 36
Megaloblasts, 67
Megalocytes, 51
Megastoma enetericum, in the feces, 292
        in the gastric contents, 251
Melanin in the urine, 516
        tests for, 516
Melanogen, 516
Melanuria, 516
Membranous dysmenorrhea, vaginal discharge in, 630
Meningeal fluid, examination of, 609
Meningococcus, 171, 616
Menstruation, vaginal discharge in, 629
Metalbumin in ovarian cysts, 619

- Metamyelocytes, 76, 81
- Methemoglobin, 37
  - sulphide, 35
- Methemoglobinemia, 37
- Methemoglobinuria, 458
- Methane. *See* Marsh gas.
- Methylene azure, 132
- Mett's method of estimating pepsin, 229
- Microblasts, 69
- Micrococcus catarrhalis, 351
  - melitensis, 174
  - tetragenus, 351
  - ureæ, 581
  - zymogenes, 171
- Microcytes, 51
- Microcythemia, 51
- Microlymphocytes, 70
- Miescher's hemometer, 152
- Milk, 633
  - chemical composition of, 633
  - cows', 633
  - examination of, 636
  - fat in, estimation of, 637
  - human, 633
  - in disease, 634
  - lactose, estimation of, 637
  - proteids of, estimation of, 637
  - secretion of, in the adult female, 633
    - in the newly born, 631
  - specific gravity of, 635
  - witches', 631
- Milk-curdling ferment in the gastric juice, 230
- Millon's reagent, 470
- Money-roll formation of red corpuscles, 53
- Monocalcium phosphate, 552
- Moro's bacillus, 280
- Motor power of stomach, examination of, 252
  - Leube's method, 252
  - salol test of Ewald and Sievers, 253
- Mouth, actinomycosis of, 202
  - secretions of, 198
  - tuberculosis of, 202
- Mucin in the feces, 322
  - in the urine, 460
- Mucor corymbifer, 353
- Mucous cylinders in the feces, 262
  - in the urine, 577
- Mucus in the feces, 262, 273
  - in the gastric contents, 246
- Müller-Weber test for blood, 247
- Murexid test, 424
- Myelemia, 116
- Myelin granules in the sputum, 336
- Myelocytes, amblychromatic, 80
  - basophilic, 82
  - eosinophilic, 81
  - neutrophilic, 76, 80
  - trachychromatic, 80
- Myelocytosis, 114

N

- Nasal catarrh, 325
  - secretion, 325
    - cerebrospinal fluid in, 325
    - characteristics of, 325
  - Charcot-Leyden crystals in, 325
    - in disease, 325
- Neisser, gonococcus of, 603
- Neisser's stain, 205
- Nematodes, 305
- Nessler's reagent, 250
- Neusser's granules, 76
- Neutral dyes, 127
  - phosphate of calcium in the urine, 558, 559
  - sulphur in urine, 396
- Neutrophilic karymorphism, 101
  - leukocytes, 74
- Nitric acid test for albumin, 462
  - oxide hemoglobin, 35
- Nitrites in the saliva, 199
- Nitrogen in the urine, 401
  - estimation of, 413
    - according to Kjeldahl, 413
- Nitrogenous equilibrium, 402
  - metabolism, 401
- Nitroprusside of sodium, as a test for acetone. *See* Legal's test.
- Normal urobilin, 499
- Normoblasts, 65
- Nose, secretion from, 325
- Nucleated red corpuscles, 65
- Nucleo-albumin in the urine, 460
  - test for, 472
- Nucleohiston in the urine, 461
- Nummular sputum, 329
- Nylander's test for sugar, 484

O

- Obermayer's reagent, 503
- Obermeier, spirochete of, 189
- Occult bleeding, 247
- Oidium albicans, 202
- Oligochromemia, 31
- Oligocythemia, 31, 57
- Oliguria, 365
- Opsonins, 639
  - Simon's index, 643
  - technique, 642
  - Wright's index, 643
- Orcin test for pentoses, 497
- Organized sediments of the urine, 561
- Ott's test, 472
- Ovarian cysts, 619, 621
- Oxalate of calcium crystals in the sputum, 551
  - in the urine, 551
- Oxalic acid, diathesis, 438
  - in the urine, 436
  - properties of, 438
  - quantitative estimation of, 439

- Oxalic acid, tests for, 439
- Oxaluria idiopathica, 438
- Oxaluric acid, 436
- Oxyamygdalic acid, 536
- Oxybutyric acid, β, in the urine, 533
    - estimation of, 533
- Oxyhemoglobin, 29
- Oxyphilic leukocytes, 77
- Oxyuris vermicularis, 307
- Ozena, 325

P

- Pancreatic cysts, 622
    - trypsin in, 622
  - juice in the gastric contents, 246
- Pappenheim's methyl-green pyronin, 595
- Paragonimus Westermanni, 343
- Paramœba hominis, 289
- Paramecium coli, 293
- Paramucin, 619
- Parasites in the blood, 177
  - in the feces, 285
  - in the gastric contents, 249
  - in the sputum, 339
  - in the urine, 580
  - malarial, 177
- Paratyphoid fever, bacillus of, 165
    - in the blood, 165
    - in the urine, 585
- Paraxanthin in the urine, 427
- Patein's albumin, 454
    - test for, 499
- Pentoses in the urine, 496
    - tests for, 497
- Pepsin in the gastric juice, 227
    - estimation of, 229
    - tests for, 229
- Pepsinogen in the gastric juice, 227
    - estimation of, 230
    - tests for, 229
- Peptones in the blood, 40
  - in the urine, 457
  - test for, 470
- Peptonuria, 457
- Pertussis bacillus, 350
- Pettenkofer's test, 320
- Pfeiffer-Widal reaction, 160
- Phagocytes, 69
- Phagocytosis, 69, 186, 639
- Pharyngomycosis leptothrica, 203
- Phenol, 517
  - estimation of, 517
  - in the feces, 318
  - in the urine, 517
  - tests for, 318, 517
- Phenylcyanate method of isolating diamins, 545
- Phenylglucosazone, 486
- Phenylhydrazin test for sugar, 486
- Phlogocytes, 82
- Phloroglucin test for pentoses, 497
  - vanillin test for hydrochloric acid, 220
- Phosphates in the urine, 382
    - estimation of, 387
    - removal of, from urine, 390
    - separate estimation of alkaline and earthy, 390
    - tests for, 386
- Phosphatic diabetes, 384
  - sediments in the urine, 558
- Picric acid test for albumin, 466
- Piria's test for tyrosin, 556
- Plague bacillus, 175, 351
- Plaques, 118
  - enumeration of, 144
- Plasma of the blood, 17, 24
- Plasmodium malariæ, 178
    - crescentic bodies, 181
    - flagellate bodies, 183
    - gametes, 182
    - hyaline bodies, 178
    - macrogametes, 184
    - merozoites, 180
    - microgametes, 184
    - microgametocytes, 184
    - oökinetes, 184
    - ovoid bodies, 181
    - pigmented extracellular bodies, 182
        - intracellular bodies, 179
    - polymites, 183
    - schizogony of, 181
    - segmenting bodies, 180
    - spherical bodies, 181
    - sporogony, 184
    - sporozoites, 184
    - staining of, 178
- Pneumococcus, 348
- Pneumoconioses, 357
- Pneumonia, diplococcus of, 348
    - in the blood, 166
  - sputum in, 348
- Pneumonomycosis aspergillina, 354
- Poikilocytes, 52
- Poikilocytosis, 52
- Polarimeter, 494
- Polarimetric test for sugar, 487, 494
- Pole bacillus, 350
- Polychromasia, 60
- Polychromatophilia, 60
- Polycythemia, 56
- Polymastigina, 290
- Polypeptids in the urine, 470
- Polyuria, 363
- Proleukocyte, 81
- Promyelocyte, 80
- Propepsin, 227
- Prostatic fluid, 624
- Proteus vulgaris, 281
- Protozoa, 286
  - in pus, 601
  - in the blood, 177

Protozoa in the feces, 286
    in the gastric contents, 251
    in the sputum, 339
    in the urine, 580
Pseudocasts, 576
Pseudodiphtheria bacillus, 206
Pseudogonococci, 603
Pseudomucin, 619
Ptomains in the feces, 323
    in the urine, 542
        isolation of, 543
Ptyalin, 199
    test for, 199
Purin, 418
    bases, in the feces, 322
        in the urine, 427
Pus, 598
    bacteria in, 600
    casts, 565
    chemistry of, 598
    corpuscles, in the urine, 567
            enumeration of, 567
    crystals in, 602
    detritus in, 600
    examination of, 602
    general characteristics of, 598
    giant corpuscles in, 600
    gonorrheal, 602
    in the feces, 271
    in the gastric contents, 248
    in the urine, 563
    leukocytes in, 599
    microscopic examination of, 599
    parasites in, 600
    protozoa in, 601
    red corpuscles in, 600
    tests for, 564
Putrescin, 543
Pyroplasma hominis, 191
Pyuria, 563

Q

Quevenne's lactodensimeter, 635

R

Ray fungus, 351
Red blood corpuscles, 51
        anemic degeneration of, 61
        behavior toward aniline dyes, 60
        enumeration of, 140
        granular degeneration of, 61
        nucleated forms, 65
        variations in color, 53
            in form, 51
            in number, 54
            in size, 51
Relapsing fever, spirochete of, 189
Resorcin test, 221
Resorptive power of the stomach, examination of, 253
Reynold's test for acetone, 529
Rhamnose in urine, 496
Rhizopoda, 286
Riegel's test dinner, 210
Ring bodies of Cabot, 64
Romanowsky's method of staining, 132
Rosenbach's reaction, 506
    test for bile pigments, 512
Ross' dehemoglobinizing method, 177
Round-worms, 305

S

Saccharimeter of Einhorn, 492
    of Lohnstein, 492
    of Soleil-Ventzke, 494
Saccharomyces cerevisiæ. *See* Yeast.
Sahli's desmoid reaction, 255
    estimation of free HCl, 223
    hemoglobinometer, 152
Saliva, 198
    chemistry of, 198
    general characteristics of, 198
    in special diseases of the mouth, 202
    in the gastric contents, 246
    microscopic examination of, 199
    nitrites in, 199
    pathological alterations of, 201
    ptyalin in, 199
    test for nitrites, 199
        for ptyalin, 199
        for sulphocyanides, 199
Salivation, 201
Salkowski's method of estimating oxalic acid in urine, 440
            xanthin bases in urine, 427
    test for albumoses, 469
        for phenol, 517
Salkowski-Volhard method of estimating the chlorides in urine, 377
Salol test of Ewald and Sievers, 253
Salzer's test meal, 210
Sand, intestinal, 264
Sarcina pulmonalis, 354
    urinæ, 586
    ventriculi, 250
Scarlatina, pharyngeal secretion in, 208
Schauffler's methylene-blue-pyronin, 206
Scherer's test for leucin, 556
Schizomycetes in the feces, 276
Schmalz and Peiper's method of determining the specific gravity of the blood, 19
Schmidt's fecal fermentation test, 268
Schüffner's stippling, 64
Sediments in acid urines, 546
    in alkaline urines, 558
    urinary, 546
        ammoniomagnesium phosphate in, 560
        ammonium urate in, 560
        amorphous urates in, 550

Sediments, urinary, bacteria in, 580
  basic magnesium phosphate in, 558, 559
  bilirubin in, 557
  blood corpuscles, red, 567
  brick-dust, 549
  calcium carbonate in, 560
    oxalate in, 551
    sulphate in, 553
  cylindroids, 576
  cystin in, 553
  epithelial cells in, 561
  fat in, 557
  foreign bodies in, 630
  hematoidin in, 557
  hippuric acid in, 552
  indigo in, 561
  leucin in, 554
  leukocytes in, 563
  mode of examination of, 548, 571
  monocalcium phosphate in, 552
  mucous cylinders, 577
  neutral calcium phosphate in, 559
  non-organized, 549
  organized, 561
  parasites in, animal, 586
    vegetable, 580
  protozoa in, 586
  soaps of lime and magnesium in, 557
  spermatozoa in, 579
  tube casts in, 570
    tumor particles in, 587
  tyrosin in, 554
  urates in, 550, 560
  uric acid in, 549
  xanthin in, 556
Semen, 624
  chemistry of, 624
  general characteristics of, 624
  microscopic examination of, 624
  pathology of, 624
  recognition of, in stains, 625
  spermatic crystals in, 624
  spermatozoa in, 624
Sepsis, organisms in the blood in, 158
Septic factor, Simon's, 90, 108
Serosamucin, 597
Serous exudates, 591
Serum albumin in the blood, 28
    in the urine, 441
      estimation of, 467
      tests for, 466
  globulin in the blood, 28
    in the urine, 454
      estimation of 469
      test for, 469
Shiga's bacillus, 276
Siderosis, 358
Simon's counting chamber, 137, 139
Simon's opsonic index, 643
  septic factor, 90, 108
  ureometer, 409
Skatol in the feces, 318
    tests for, 319
Sleeping sickness, organism of, 188
Slides, 120
Small mononuclear leukocytes, 70
Smegma bacillus, 351, 583
Smith's test for bile pigment, 512
Soaps of lime and magnesium in the urine, 557
Sodium chloride in hydatid fluid, 621
Spermatic crystals, 623
Spermatocystitis, 580
Spermatorrhea, 580
Spermatozoa in the semen, 624
  in the urine, 579
Spermin, 624
Spiegler's reagent, 466
Spirals of Curschmann, 332
Spirilla of Vincent's angina, 204
Spirochæte Obermeieri, 189
  pallida, in blood, 191
    in syphilitic material, 606
Splenocytes, 73
Sporozoa, 294
Spotted fever, organism of, 191
Sputum, 326
  Amœba coli in, 339
  amount of, 327
  bacteria in, 344
  blastomycetes in, 353
  blood in, 335
  cheesy particles in, 330
  chemistry of, 358
  color of, 328
  concretions in, 333
  configuration of, 329
  consistence of, 327
  crudum, 329
  crystals in, 354
  Curschmann's spirals in, 332
  Diplococcus pneumoniæ in, 348
  Distoma pulmonale in, 343
  echinococcus in, 333
  elastic tissue in, 330, 337
  epithelial cells in, 336
  fibrinous casts in, 330
  foreign bodies in, 333
  general characteristics of, 326
  globosum, 329
  heterogeneous, 329
  homogeneous, 329
  influenza bacillus in, 349
  leukocytes in, 334
  macroscopic constituents of, 330
  microscopic examination of, 334
  nummular, 329
  odor of, 328
  parasites, animal, in, 339
    vegetable, in, 344
  specific gravity of, 329

Sputum, streptothrices in, 351
  Tænia echinococcus in, 339
  technique in the examination of, 326
  tubercle bacillus in, 344
Staining, methods of, 129
  principles of, 125
Staphylococcus pyogenes albus, 169
    aureus, 169
    citreus, 169
Steatorrhea, 262
Stercobilin, 320, 513
Stercoraceous material in the vomit, 248
Stimulation forms, 82
Stomach, motor power of, 252
  rate of absorption in, 254
  tube, 211
    contra-indications to its use, 211
    its introduction, 211
  washing, 212
Stomatitis, catarrhal, 202
  gonorrheal, 202
  ulcerative, 202
Stools. *See* Feces.
Strauss' test for lactic acid, 236
Strecker's test for xanthin, 557
Streptococcus pyogenes, 170
    brevis, 170
    conglomeratus, 170
    longus, 170
Streptothrices, 351
Streptothrix actinomycotica, 352
      eppingeri, 352
      hominis, 352
      pseudotuberculosa, 352
Strongyloides, 308
    intestinalis, 312
Strongylus duodenalis, 308
Stycosis, 358
Succinic acid in hydatid fluid, 621
Sudan stain for fat, 572
Sugar in the blood, 40
  in the urine, 474
    estimation of, 489
    tests for, 483
Sulphanilic acid test. *See* Ehrlich's reaction.
Sulphates in the urine, 390
    estimation of total, 394
  conjugate, 392
  estimation of, 395
  mineral, 391
  tests for, 394
Sulphocyanides, in the saliva, 199
  in the urine, 396
Sulphohemoglobin, 35
Sulphur, neutral, in urine, 396
      estimation of, 398
Syphilitic blood test of Justus, 32
Syphilis. *See* Spirochæte pallida.

T

Tænia Africana, 299
  canina, 299
  cucumerina, 299
  diminuta, 299
  echinococcus, 339
  flavopunctata, 299
  lata, 300
  Madagascariensis, 299
  mediocanellata, 295
  nana, 297
  saginata, 295
  solium, 297
Talquist's hemoglobinometer, 152
Tartar, 203
Taurocarbaminic acid in urine, 396
Teichmann's crystals, 36
Test breakfast of Boas, 210
    of Ewald and Boas, 210
  dinner of Riegel, 210
  meal of Salzer, 210
  meals, 210
Thiosulphates in urine, 396
Thoma-Zeiss' hemocytometer, 140
Thrush, 202
Toison's fluid, 140
Tollen's orcin test, 497
  phloroglucin test, 497
Tongue, coating of, 203
Tonsillitis, 204
Tonsils, coating of, 203
Töpfer's method of estimating hydrochloric acid, 222
  test for hydrochloric acid, 219
Transition forms, 74
Transudates, 588
  albumin in, 589
  chemistry of, 590
  coagulation of, 590
  general characteristics of, 588
  microscopic examination of, 591
  specific gravity of, 588
Trematodes, 302
Treponema pallidum, in blood, 191
    in syphilitic material, 606
Triacid stain, Ehrlich's, 130
    Pappenheim's, 126
Trichina spiralis, 311
Trichloracetic acid test, 465
Trichocephalus dispar, 311
Trichomonads in the feces, 291
  in the sputum, 339
  in the stomach contents, 251
  in the urine, 586
  in vaginal discharges, 628
Trichomonas vaginalis, 291
Trichotrachelides, 311
Triple phosphate crystals in the sputum, 357
      in the urine, 560
Tripperfäden, 566
Trommer's test, 483

- Tropeolin test for hydrochloric acid, 221
- Trypanosoma gambiense, 188
- Trypanosomiasis, 187
  - in the blood, 187
  - in the cerebrospinal fluid, 618
- Trypsin in pancreatic cysts, 622
  - test for, 622
- Tube casts in the urine, 571
  - amyloid, 575
  - blood, 572
  - clinical significance of, 577
  - compound hyaline, 573
  - epithelial, 572
  - fatty, 574
  - granular, 574
  - hyaline, 572
  - mode of examination of, 571
  - pseudo-, 576
  - pus, 565
  - staining of, 572
  - true, 572
  - waxy, 574
- Tubercle bacillus, 344
  - cultivation of, 347
  - detection of, 345
  - in the blood, 173
  - in the cerebrospinal fluid, 616
  - in the feces, 285
  - in the milk, 634
  - in the mouth, 202
  - in the sputum, 344
  - in the urine, 582
  - staining of, 345
- Tuberculosis, bacillus of, 344
- Tumor particles in the gastric contents, 252
  - in the urine, 587
- Türck's counting chamber, 140
- Typhoid fever, bacillus of, 159, 278
  - in the blood, 159
  - in the feces, 278
  - in the sputum, 351
  - in the urine, 584
- Typhus fever, apiosoma of, 190
- Tyrosin, in the sputum, 357
  - in the urine, 538, 554
  - test for, 556

U

- UFFELMANN's test for lactic acid, 235
- Ulceromembranous angina of Vincent, 204
- Uncinaria Americana, 308
  - duodenalis, 307
- Unna-Tänzer stain, 339
- Urates in urinary sediments, 550, 560
- Urea in the blood, 42
  - in the urine, 399
    - estimation of, 409, 412
    - origin of, 399
    - properties of, 407
    - tests for, 407
- Urea nitrate, 408
  - oxalate, 408
- Uremia, 43
- Ureometers, 409
  - Doremus', 411
  - Heinz, 411
  - Simon's, 409
- Uric acid, 417
  - crystals of, 549
  - diathesis, 421
  - estimation of, 424
    - Folin's method, 424
    - Hopkins' method, 424
    - Ludwig-Salkowski method, 427
  - in blood, 43
  - in sediments, 588
  - in urine, 417
  - properties of, 423
  - tests for, 424
- Urine, 360
  - acetone in, 527
  - acidity of, 371
  - albumins in, 440
  - albumoses in, 454
  - alkapton in, 518
  - alloxur bases in, 427, 556
  - amino-acids in, 538
  - ammonia in, 415
  - amount, 362
  - animal parasites in, 586
  - bacteria in, 580
  - Bence Jones' albumin in, 456
  - benzoic acid in, 431
  - bile acids in, 512
    - pigments in, 510
  - blood in, 567
    - blue, 521
  - carbohydrates in, 474
  - carbonates in, 560
  - casts in, 570
  - chemistry of, 373
  - chlorides in, 374
  - cholesterin in, 513
  - chromogens in, 501
  - chyle in, 539
  - color of, 360
  - consistence of, 362
  - crotonic acid in, 535
  - cystin in, 397, 553
  - dextrin in, 496
  - diacetic acid in, 532
  - Ehrlich's diazo reaction, 522
    - benzaldehyde reaction, 526
  - epithelium in, 561
  - fat in, 539, 557
  - fatty acids in, 537
  - ferments in, 540
  - fibrin in, 459
  - foreign bodies in, 587
  - gases in, 541
  - general appearance of, 360
    - chemical composition of, 373

Urine, glucose in, 474
  glucuronic acid in, 497
  green, 521
  hematoporphyrin in, 508
  hemoglobin in, 458
  hippuric acid in, 429, 552
  histon in, 461
  homogentisinic acid in, 518
  indican in, 501
  indigo in, 561
  inosit in, 498
  kreatin in, 432
  kreatinin in, 432
  kryoscopy in, 546
  lactic acid in, 535
  lactose in, 495
  laiose in, 496
  leucin in, 554
  leukocytes in, 563
  levulose in, 495
  maltose in, 496
  melanin in, 516
  microscopic examination of, 546
  mineral ash, estimation of, 373
  neutral sulphur in, 396
  nitrogen in, 402
  nucleo-albumin in, 460
  nucleohiston in, 461
  odor of, 362
  organized sediments in, 561
  oxalates, 551
  oxalic acid in, 436
  oxaluric acid in, 436
  oxyamygdalic acid, 536
  oxybutyric acid in, 533
  parasites in, 580
  pentoses in, 496
  peptone in, 457
  phenol in, 517
  phosphates in, 382, 552, 558
  pigments in, 498
    referable to drugs in, 521
  ptomains in, 542
  pus in, 563
  quantity of, 362
  reaction of, 369
  sediments in, 546
  serum albumin in, 441
    globulin in, 454
  solids in, 368
  specific gravity of, 366
  spermatozoa in, 579
  sugar in, 474
  sulphates in, 390
  sulphur neutral in, 396
  tube casts in, 570
  tumor particles in, 587
  tyrosin in, 554
  urates in, 550, 560
  urea in, 399
  uric acid in, 417, 549
  urobilin in, 513
  urochrome in, 499
Urine, uroerythrin in, 500
  urohematin in, 506
  urohematoporphyrin in, 508
  urorosein in, 507
  vegetable parasites in, 580
  xanthin bases in, 427, 556
Urines, blue, 521
  green, 521
Urinometers, 367
Urobilin, febrile, 513
  in the blood, 49
  in the urine, 513
  normal, 499
  pathological, 513
  tests for, 49
    Braunstein's, 515
    Gerhardt's, 515
    Schlesinger's, 515
    spectroscopic, 515
Urobilinogen, 513
Urobilinuria, 513
Urochrome, 499
Uroerythrin, 500
Urofuscohematin, 508
Urohematin, 506
Urohematoporphyrin, 508
Urorosein, 507
Uroroseinogen, 507
Urorubrohematin, 508

V

VAGINAL blennorrhea, 628
  discharges, 627
    bacteria in, 627
    during menstruation, 629
    following parturition, 629
    general description of, 627
    in abortion, 631
    in gonorrhea, 630
    in membranous dysmenorrhea, 630
    in uterine cancer, 630
    in vaginitis, 629
    in vulvitis, 629
    parasites in, 628
    reaction of, 627
Vaginitis exfoliativa, 630
Vaughan's granules, 64
Vincent's angina, 204
  fusiform bacillus, 204
Vitalli's test for pus, 564
Volume index, 147
Vomited material, 244
    bile in, 246
    blood in, 247
    food material in, 244
    mucus in, 245
    odor of, 249
    pancreatic juice in, 246
    parasites in, 249
    pus in, 248

Vomited material, saliva in, 246
    stercoraceous material in, 248
Vomitus matutinus, 246
Von Fleischl's hemometer, 150
Vournaso's test for lactic acid, 236

W

Wang's estimation of indican, 504
Waxy casts, 574
Weigert-Ehrlich stain, 346
Weigert's elastic-tissue stain, 339
Weyl's test for kreatinin, 434
Whetstone crystals. *See* Uric acid.
White blood corpuscles. *See* Leukocytes.
Whooping-cough, bacillus of, 350
Widal's serum test, 160
Williamson's blood test in diabetes, 42
Worms. *See* Vermes.
Wright's coagulometer, 28
    stain, 135

X

Xanthin bases in the blood, 44
    in the feces, 322
    in the urine, 418, 427, 556
        estimation of, 427
Xylose in urine, 496

Z

Zappert-Ewing counting chamber, 140
Ziehl-Neelsen stain, 346
Zymogens in the gastric juice, 227